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ON THE TISSUE PHASE OF THE LIFE CYCLE OF THE FOWL NEMATODE *ASCARIDIA GALLI* (SCHRANK)[1, 2]

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INTRODUCTION AND REVIEW OF LITERATURE

A migratory phase in the life cycle of an ascarid was discovered by Stewart (1916) who fed eggs of *Ascaris lumbricoides* to mice and found larvae in their livers and lungs. Such a larval migratory phase for *A. lumbricoides* was confirmed by Ransom and Cram (1921) with guinea pigs as hosts. Danheim (1925) demonstrated that the larvae of four other species of nematodes belonging to the family ASCARIDIDAE have a migratory habit.

Ackert (1923) found that larvae of the fowl nematode *Ascaridia galli*[4] (Plate I, fig. 1) (HETERAKIDAE) bury their anterior ends deeply between the intestinal villi and into the glands of Brunner (Plate I, fig. 2), but that they seldom pass through the wall of the intestine or migrate over the body of the host. Similar findings were reported by Roberts (1937) for these phases of the life cycle of *A. galli*.

Dorman (1928) found larvae of *Heterakis gallinae* in all parts of the lumina of the caeca but not in the viscera. Clapham (1933) confirmed this observation, showing that *H. gallinae* develops without migrating into the tissues. At about the same time, Baker (1933) stated that from inoculation to about the fourth day there was a rather close association of this nematode with the mucosa of the caecum during which time injury to the glandular epithelium may occur.

The presence of *A. galli* larvae in the intestinal mucosa was announced by Ackert (1923). Death of chickens with heavy infections of *A. galli* was attributed by Guberlet (1924) to inflammation of the intestine. He stated that heavily infected birds died from intestinal irritation and toxemia on the 10th to 12th day following inoculation. Ackert and Herrick (1928) found that the most deleterious period in the life cycle of the worms was about the 14th day after the embryonated eggs were swallowed by chicks a month or less of age. The symptoms of weakness, etc. exhibited by heavily infected chicks were most marked during the 10th to the 17th day, thus suggesting that the majority of worms were in the mucosa during that period. It was later determined by Ackert (1931) that these symptoms coincided with the

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[4] Synonyms, *Ascaridia perspicillum* and *A. lineata*.

tissue phase of this nematode. However, the exact beginning and end of the tissue phase was undetermined.

An apparatus for flushing *A. galli* from the intestinal lumen with warm water under pressure was developed by Ackert and Nolf (1929), but the collection of young ascarids from the intestinal wall hitherto has been an unsolved problem. Efforts were begun by Ackert and Tugwell (1948) to attack this problem by application of a mass digestive apparatus similar to that used by investigators for conducting surveys on the prevalence of trichinosis. The use of this technique eliminated time-consuming micro-sections and teasing methods formerly used for this purpose.

MATERIALS AND METHODS

Chickens

White Leghorns were used because this breed was found to be one of the most susceptible to *A. galli* infections and consequently better for comparison of parasitic infections (Ackert and Wilmoth, 1934; Ackert, Eisenbrandt, Wilmoth, Glading and Pratt, 1935). All chickens used in this study were obtained as day old chicks from commercially approved hatcheries and raised helminth-free until inoculated. Throughout the experiments all chickens were given the same ration and kept under similar conditions of care and environment.

Egg Cultures

The *A. galli* eggs, which were obtained from adult female worms collected from freshly killed chickens, were cultured in the ascarid uteri in Petri dishes as recommended by Riedel (1947) and subsequently were fed to the chickens by Riedel's drop method.

Egg doses of 100 or 200 were shown by Ackert, Graham, Nolf and Porter (1931) and later by Ackert, Cooper and Dewhirst (1947) to be suitable for comparative fowl ascarid studies, since the average number of worms obtained per chicken was comparable to the average infection of 1,000 chickens examined in the vicinity of Manhattan, Kansas (Ackert, 1930).

Collection of Larvae

Flushing. The collection of the larvae from the lumen (lumen-larvae) was accomplished through the use of the hydraulic method of Ackert and Nolf (1929). Each chicken was killed, and the intestine from the gizzard to the yolk sac diverticulum was removed, stripped of mesenteries and divided into lengths of approximately 30 cm.[5] They were then quickly flushed out with warm water into an Erlenmeyer flask. According to Ackert, Edgar and Frick (1939) the mucus accumulation was negligible when the operation was performed quickly, and the free worms were flushed into jars. The worms were allowed to remain in the intestinal debris for approximately 12 hours to straighten out and die. They were then counted and preserved in vials containing 10 per cent formalin.

Digesting. The artificial digestion apparatus which was assembled by the senior author[6] included a constant temperature water bath within an insulated box with

[5] Ackert (1923; 1931) showed that the normal habitat of this nematode is in the duodenum a few centimeters behind the entrance of the bile ducts.

[6] The apparatus is essentially similar to one perfected by Dr. J. Henry Walker of the University of Alabama.

heat provided by standard electric light bulbs spaced around the water bath and controlled by a thermoregulator. The freshly flushed intestines were digested in quart jars containing a 0.5 per cent solution of hydrochloric acid and 1.0 per cent pepsin to free larvae from the intestinal mucosa (mucosa-larvae). Constant agitation was obtained with glass stirring rods rotated by a system of pulleys and belts driven by an electric motor. The contents of the quart jars were agitated from three to four hours depending upon the volume of the intestine to be examined. At the end of this period the jars were removed from the digesting device and the worms allowed to settle to the bottom. The supernatant fluid was drawn down to a three-centimeter depth with a J-shaped glass tube, rubber hose and a negative pressure pump attached to a standard threaded water faucet. The jar was then filled with tap water, allowed to stand and the supernatant fluid again removed to a depth of about three centimeters. Transfer of small volumes of the residue to Petri dishes and examination with a wide-field binocular microscope facilitated the isolation of the larvae which were placed in vials and preserved in 10 per cent formalin.

Measurement of Worms

Measurement of the larger worms was accomplished by projecting an image of each worm through a lens in a photographic bellows onto a screen. The mechanism magnified the size of the worm six times. A tracing of each worm was made and these tracings measured with a milled wheel having peripheral graduations six times larger than the unit used (Ackert, et al., 1935; Ackert, Whitlock, and Freeman, 1940).

Measurements of the smaller worms were made with the aid of a camera lucida. Tracings of the worms were measured with the graduated milled wheel and the length of each worm was recorded for comparison with lumen worms of corresponding ages.

EXPERIMENTAL RESULTS

Five experiments were run on groups of chickens numbering about 25 each. The ages of the respective groups of fowls when parasitized were 11, 14, 24, 38 and 60 days. Fowl groups 3, 4 and 5 were fed 200 $\pm$ 10 *A. galli* eggs per chicken, while group 1 was given 100 $\pm$ 10 and group 2, 500 $\pm$ 10 eggs per fowl.

Number of Worms

Examinations in group 1 were begun on the 8th day, somewhat in advance of the supposed time of entrance of the *A. galli* into the intestinal mucosa. Flushing yielded 1 larva (Gp 1f), digesting the intestine none (Table 1). Examinations on the 9th day showed 2 lumen-worms (Gp 1f) and 1 mucosa-larva (Gp 1d). Findings from the 10th day fowl showed 2 lumen-larvae and 18 mucosa-larvae all apparently uninjured by the digestive process.

Examinations of fowls during the rest of this experiment showed that the tissue phase was most prominent from the 10th to the 17th day, but that it extended to the 23rd day (Gp 1d). Lumen-larvae were numerous (26) on the 11th day of parasitism, and were present daily to the 20th day. That many larvae had withdrawn from the mucosa by the 21st day was indicated by the presence of 45 lumen-larvae to 1 mucosa-larva (Table 1).

TABLE 1.—*Summary of data on numbers and average lengths of the fowl nematode* A. galli *from each chicken in 5 experiments to determine the tissue phase of the life cycle of this nematode*[7]

Days	Number of worms collected from chickens										Average lengths (mm.) of worms										Over-all avg. lengths (mm.) of worms	
Inoc. to exam. of chick	Flushing					Digesting					Flushing					Digesting					Flushing	Digesting
	Gp 1f	Gp 2f	Gp 3f	Gp 4f	Gp 5f	Gp 1d	Gp 2d	Gp 3d	Gp 4d	Gp 5d	Gp 1f	Gp 2f	Gp 3f	Gp 4f	Gp 5f	Gp 1d	Gp 2d	Gp 3d	Gp 4d	Gp 5d		
1	..	0	0	0	2	..	0	1	0	0					0.3			0.3			0.3	0.3
2	..	17	2	0	1	..	0	2	0	0		0.7	0.3		0.3			0.3			0.7	0.3
3	..	21	0	0	0	..	0	8	3	0								0.6	0.4			0.6
4	..	3	0	0	1	..	0	3	3	6					0.4			0.8	0.8	0.6	0.4	0.7
5	..	5	0	0	0	..	2	14	4	0							1.0	1.0	0.9			0.9
6	..	0	7	1	0	..	4	9	8	1			1.1	1.0			0.9	1.0	1.1	1.0	1.1	1.0
7	..	0	4	2	0	..	0	9	11	1			1.6	2.1				1.3	2.1	1.7	1.8	1.7
8	1	0	2	7	0	0	1	51	25	5	2.4		2.1	2.4			3.4	2.4	2.5	2.4	2.3	2.4
9	2	11	13	15	1	1	21	42	104	1	2.6	2.8	2.8	3.0	3.3	3.5	3.1	2.6	3.3	2.6	2.9	3.1
10	2	6	25	2	4	18	1	52	36	17	3.6	3.2	3.5	3.8	3.8	2.6	3.5	3.7	3.3	3.5	3.9	3.4
11	26	91	9	1	1	9	344	62	43	22	3.1	4.3	2.9	3.3	1.6	2.7	2.6	2.7	4.2	3.6	3.9	2.8
12	3	101	10	6	3	17	129	43	31	15	3.9	5.3	2.8	4.0	4.3	3.2	3.7	3.9	4.3	4.7	5.0	3.8
13	11	223	22	9	3	28	210	42	36	27	3.3	4.4	2.5	4.0	3.8	3.7	3.6	3.2	4.0	3.8	3.7	3.6
14	7	96	11	4	1	8	4	49	3	6	6.9	3.2	5.6	7.2	7.3	3.2	2.8	4.3	4.0	3.6	3.8	4.0
15	11	25	1	63	2	17	1	10	18	14	4.8	6.2	4.0	9.2	4.5	3.6	4.0	3.2	4.3	4.5	7.8	4.0
16	6	36	6	42	0	1	53	23	2	7	9.1	6.4	7.8	13.9		3.7	3.5	3.7	3.9	3.7	10.2	3.6
17	11	515	3	36	2	12	7	2	2	8	17.6	17.9	7.0	15.3	9.8	3.1	3.7	3.8	3.7	3.9	12.8	3.5
18	1	0	50	50	3	0	5	0	2	6			13.6	22.9	12.9		3.4		4.2	5.1	17.8	4.3
19	14	0	2	5	3	0	0	2	1	0	16.9		9.7	15.0	18.5			2.6	3.2		16.1	2.8
20	0	29	1	9	0	7	10	1	4	2		10.8	3.6	15.0		3.0	3.7	3.9	3.4	3.6	11.6	3.4
21	45	12	13	15	59	1	0	8	2	1	29.5	12.3	12.1		23.8	3.7		3.2	3.6	4.0	19.1	3.4
22	1	33	2	0	10	0	0	2	2	23	20.0	25.0	16.1		18.7			3.8	5.3	3.2	23.1	3.4
23	1	11	6	..	15	1	0	1	..	0	15.2	27.0	14.4		22.1			3.2			22.1	3.2
24	1	..	21	..	0	0	..	4	..	2	10.5		16.3					2.2		4.1	16.0	2.8
25	5	..	8	..	34	0	..	7	..	1	36.5		14.1		24.6			3.3		26.1	24.1	3.3
26	1	..	1	..	..	0	..	2	..	..	4.3		13.6					3.1			13.6	3.1

[7] In assembling data from 5 experiments to make this table, it was necessary to omit the data from the duplicate examinations of chickens in each experiment—a total of 42 fowls which harbored 684 *A. galli*. The duplicate examinations which occurred from the 10th to the 23rd days were mostly from the 10th to the 14th day. Omission of these data on numbers and lengths of the *A. galli* did not change the nature of the results of the study as shown in text-figures 1 and 2.

In the 2nd and in all the remaining experiments, examinations of chickens began on the 1st day after inoculation with the *A. galli* eggs. Results from group 2 showed only lumen-larvae up to the 5th day when 2 mucosa-larvae were found. The 9th day fowl had 11 lumen-larvae and 21 mucosa-ones. Heavy infections from this group of fowls fed 500 ± 10 *A. galli* eggs were found first in the 11th day chicken which had 91 lumen-worms and 344 mucosa-larvae (Gp 2d). The 12th and 13th day fowls also had heavy infections in both the lumina and the mucosae. Mucosal infections dropped off markedly after the 13th day, but the numbers of lumen-larvae were considerable to the 17th day when they reached their maximum of 515 worms (Gp 2f).

The tissue phase in the life cycle of *A. galli* was manifested from the first day in the 3rd experiment when 1 mucosa-larva was found (Gp 3d). Substantial numbers of mucosa-larvae were found after digesting the flushed intestines during the first week, while lumen-larvae were absent or scarce. Fowls examined from the 8th to the 16th day yielded sizable infections of mucosa-larvae (10–62), and constant, but somewhat smaller numbers of lumen-larvae (1–25). The latter were more numerous from the 18th day (1–50) than were the mucosa-larvae (1–8) which extended the tissue phase into the 26th day of parasitism.

The heavier infections in the 4th experiment were those of the tissue phase from the 8th to the 15th day when a maximum of 104 mucosa-larvae were collected on the 9th day. A maximum of 63 lumen-larvae isolated on the 15th day initiated a week of fairly heavy infections. The lumen-larvae continued rather numerous until the 21st day, while the mucosa-larvae dwindled to 2 on the 22nd day (Gp 4d).

In the 5th and last experiment which was upon somewhat older chickens, the tissue phase was apparent first on the 4th day (Gp 5d). The heaviest period of this phase was from the 10th to the 15th day, while that for the lumen-worms was from the 21st to the 25th day (Gp 5f). Evidence of age resistance is apparent from the smaller numbers of *A. galli* collected from both the lumina and the mucosae of this group of chickens than from the 2 previous groups which were 3 to 5 weeks younger.

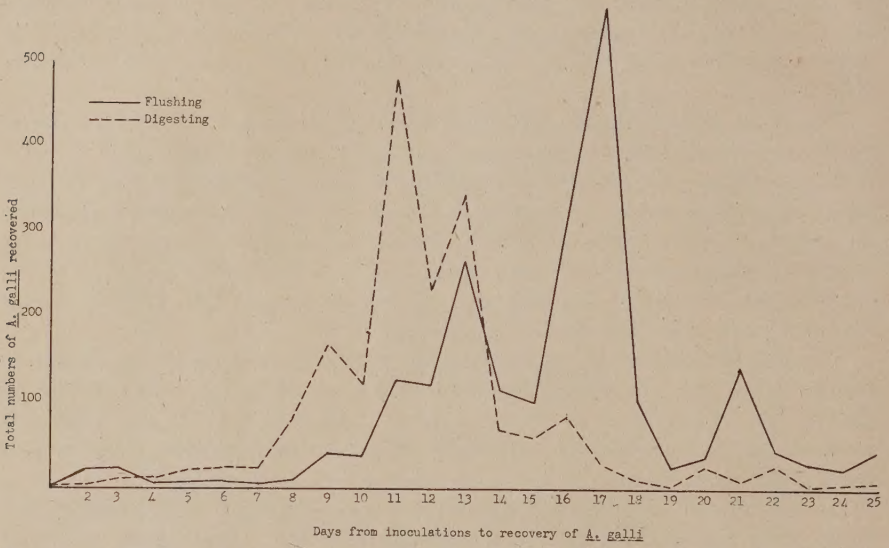
The general pattern of the life cycle of *A. galli* as found by this study is that the height of the tissue phase precedes that of the lumen phase. From text-figure 1 it is seen that while the tissue phase may begin on the 1st day of parasitism, its intensity begins on the 7th day, reaches its peaks from the 9th to the 16th days and may continue through the 25th day. The lumen phase which also may begin on the 1st day reaches its peaks from the 11th to the 21st days.

Growth of Worms

In determining the growth of the *A. galli,* measurements of the length of each worm in millimeters was made. Upon hatching, the average length of the larva was 0.3 mm. Growth of the larvae through the 6th day proceeded at approximately the same rate in the lumen as in the tissue phase (Table 1, Gp 1f–5f & Gp 1d–5d), the young worms becoming about 1 mm. in average length. Similarly, the increases in average sizes of the lumen and mucosa-larvae developed at about the same rate through the 13th day of parasitism when they were approximately 4 mm. long. The 14th day brought considerably larger lumen-larvae from flushing in 4 of the 5 groups while the mucosa-larvae remained at approximately the 4 mm. length. From

about the 15th day on, normal growth rates occurred among the lumen-larvae, but the mucosa-larvae made no further growth, at least through the 26th day of parasitism (Table 1).

The length of 26.1 mm. shown in Gp 5d (25th day) is that of a single worm collected with the mucosa-larvae. Its large size shows that it was a lumen-worm which escaped removal from the fowl intestine by flushing.



Text-fig. 1. Daily total numbers of *A. galli* collected by flushing and digesting in this study.

Comparison of Lumen and Mucosa-Larvae (Moults)

According to Chitwood and Chitwood (1940) nematodes have four moults. In *A. galli,* the 1st moult occurs in the egg before the hatching of the larva (Alicata, 1934). In the present study the 2nd moult of the lumen-larvae occurred in the 6 to 8-day period after hatching, the 3rd when 14 to 15 days of age, and the last during the 18 to 22-day period of the *A. galli* life span. The 3rd stage larvae, that is, those between the 2nd and 3rd moults, showed a sickle-shaped tail and preanal swelling (males), and anal prominences in both males and females. Characteristics of the 4th stage lumen-larvae are proportionately shorter tails in the females, and males with prominent anus and preanal sucker. After the 4th moult, adult characteristics prevailed in the young *A. galli.*

Explanation of Plate I

A. galli Larvae from Intestinal Mucosa

Fig. 1. One-day old larva. (2nd stage)

Fig. 2. Section of chicken intestine showing larva destroying Brunner's glands (After Ackert, 1923).

Fig. 3. Ten-day male larva. (Early 3rd stage)

Fig. 4. Eight-day female larva. (3rd stage)

Fig. 5. Fifteen-day male larva. (Late 3rd stage)

Fig. 6. Fifteen-day female larva. Late 3rd stage)

Fig. 7. Twenty-two-day male larva. (Late 3rd stage)

Fig. 8. Twenty-two-day female larva. (Late 3rd stage)

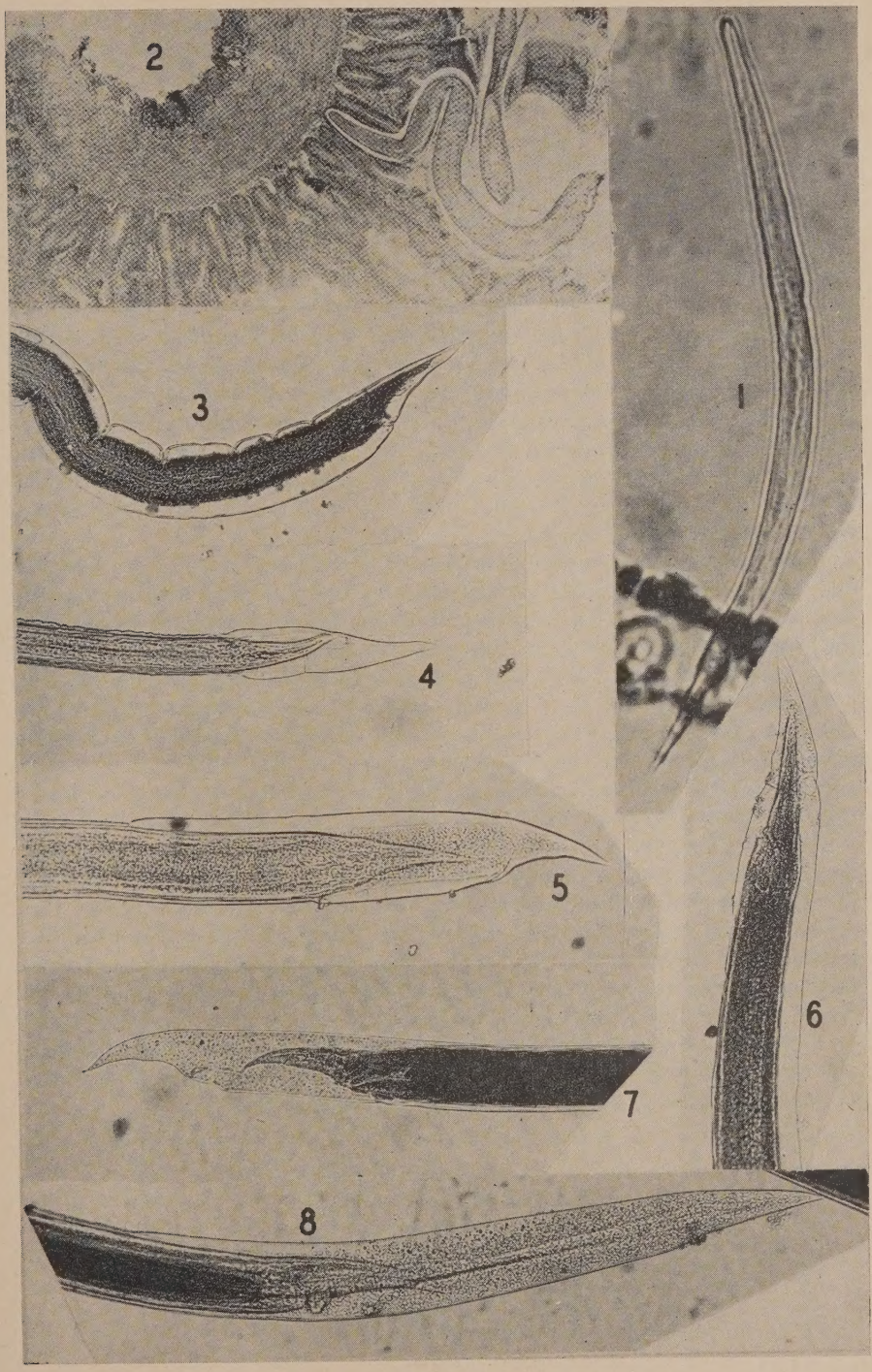


PLATE I

Plate I, figs. 3 and 4, respectively, show male and female larvae from the mucosa. The male which was 10 days old showed the typical sickle-shaped tail and the beginning of a preanal swelling. Anal prominences, although present, are not distinct. Figure 4 shows the old cuticle of the 8-day female larva distinctly separated from the new.

A male mucosa-larva isolated on the 15th day is shown in Plate I, fig. 5. Characteristic is the short sickle-shaped tail and the preanal swelling which is evident on the old cuticle. Also evident is the anal prominence on the old cuticle, but there is no indication of the preanal sucker on the worm within the cuticle. The female larva (Plate I, fig. 6) shows the slightly longer tail. The anal prominence is very evident on the worm within the old cuticle.

Plate I, figs. 7 and 8, illustrate larvae isolated from the intestinal mucosa during the 22nd day. The old cuticle (fig. 7) shows the characteristics of the 3rd stage larva, namely, short sickle-shaped tail, anal prominence and preanal swelling. On the new cuticle, the characteristics of the 4th stage *A. galli* male are evident, especially the beginning of the preanal sucker. Figure 8 shows a 22-day female mucosa-larva with the old cuticle distinctly separated from the new. Evident is the typical posterior end of the young 4th stage *A. galli*. The anal prominences on the old and new cuticles are partially obscured by debris.

The moulting of the mucosa-larvae showed a broad range of days for each moult with these periods overlapping considerably. Evidences of the 2nd moult occurred from the 8th to the 17th day. Larvae with characteristics of those recovered from the lumen after the 3rd moult and before the 4th were not found until the 14h day, while larvae in the process of the 2nd moult were found as late as the 24th day.

Figures 1 and 2 of Plate II show the posterior ends, respectively, of an 8-day male and a 10-day female larva from the lumen. These drawings show characteristics of 3rd stage larvae, namely, typical sickle-shaped male tail and a preanal swelling and the female with an anal prominence. With the exception of the latter characteristic, the lumen and the mucosa-larvae are much alike. (Plate II, figs. 3 and 4.)

Plate II, fig. 3, shows a 15-day male larva from the lumen. The loose cuticle at the posterior end clearly shows the characteristics of the 3rd stage larva, while the new cuticle shows the characteristics of the 4th stage male larva (well defined preanal sucker and anal prominence). Plate II, fig. 4 illustrates the characteristic anal prominence of the 3rd stage female larva. Plate I, figs. 5 and 6 show male and female mucosa-larvae, respectively, on the same day. It will be noted that these still retain characteristics of 3rd stage lumen larvae.

Larvae from the lumen on the 22nd day (Plate II, figs. 5 and 6) show the characteristics of 4th stage *A. galli* larvae, while those from the mucosa on the same day (Plate I, figs. 7 and 8) have characteristics of 3rd stage larvae.

EXPLANATION OF PLATE II

Lumen Larvae of *A. galli*

FIG. 1. Eight-day old male larva. (Early 3rd stage)
FIG. 2. Ten-day female larva. (Early 3rd stage)
FIG. 3. Fifteen-day male larva. (Late 3rd stage)
FIG. 4. Fifteen-day female larva. (Late 3rd stage)
FIG. 5. Twenty-two-day male larva. (4th stage)
FIG. 6. Twenty-two-day female larva. (4th stage)

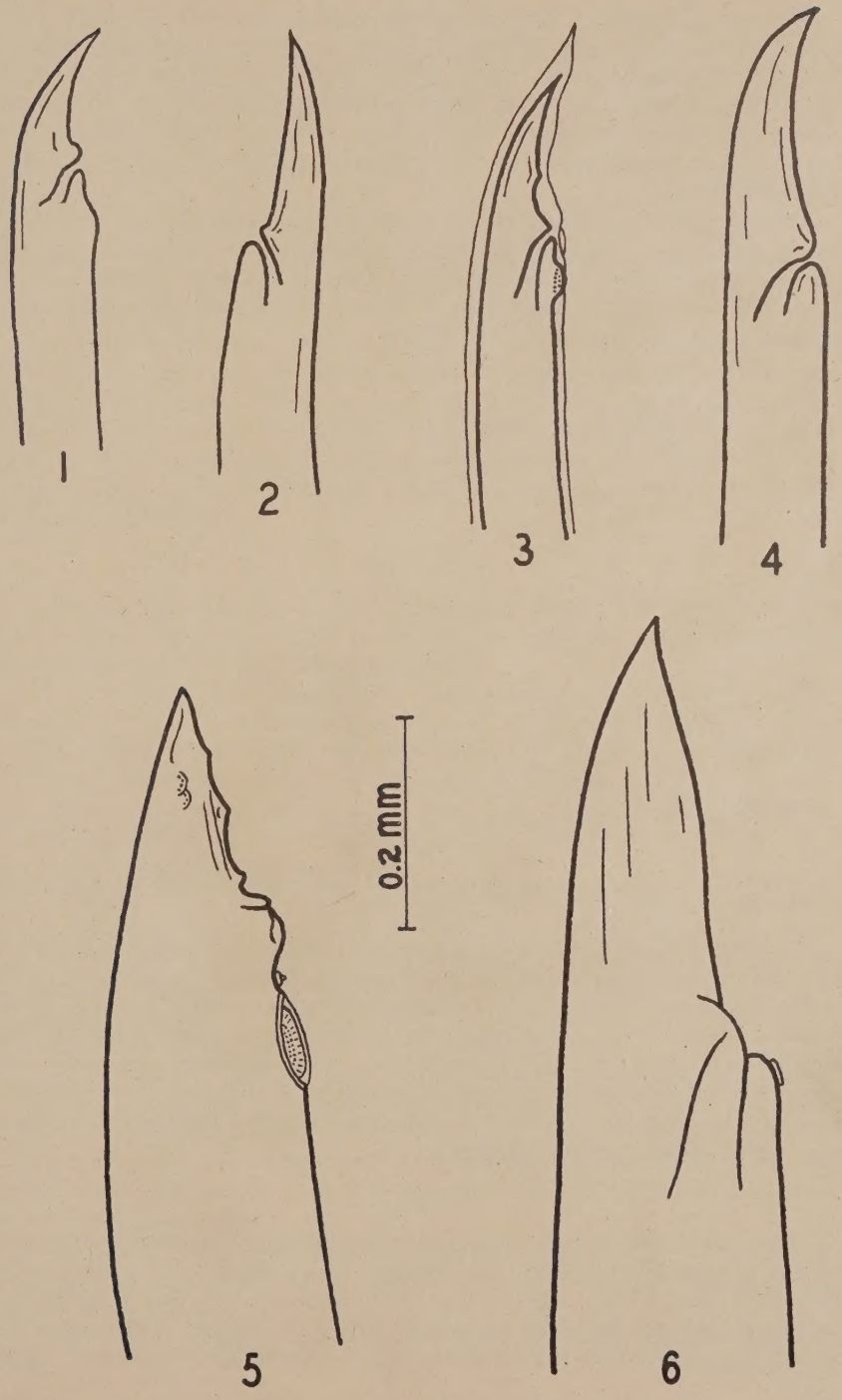
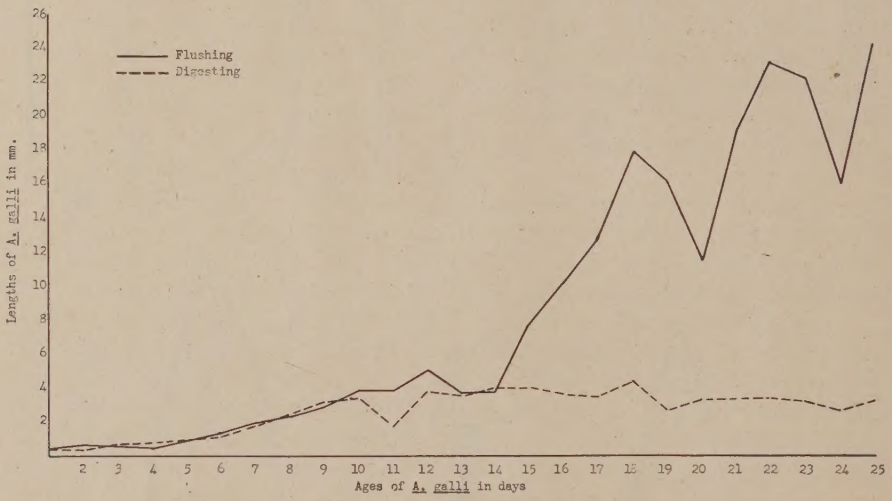


PLATE II

From the foregoing, it becomes apparent that by the time of the 4th moult (18–22nd day) of the nematodes in the lumen, the mucosa-larvae have lagged to the extent of being one moult behind. Thus, these mucosa-larvae have developed only to the 3rd larval stage. References to text-figure 2 shows that little growth occurred in the lumen-larvae until the 6- to 8-day period (2nd moult) and that there was little increase in size until the 3rd moult (14th to 16th day), after which there was normal growth.

Probably the mucosa-larvae are not structurally developed for tissue feeding or for any major growth. Apparently, they do not complete the 3rd moult while they are in the intestinal mucosa. Ackert (1938), who failed to obtain growth in *A. galli* larvae introduced into the body cavity of fowls, inferred that these larvae are not equipped to feed on lymph and other tissues.



Text-fig. 2. Comparison of average lengths of *A. galli* recovered by flushing and digesting in this study.

Failure of the mucosa-larvae to make growth comparable with that of the lumen-larvae affords a plausible explanation of the occasional presence of diminutive larvae in lumen flushings. Ackert and his associates have been plagued for years by the presence of such larvae in flushings from the intestines of experimental chickens. Heretofore, the presence of these dwarfed larvae, which doubtless were shaken loose from their mucosa moorings in the flushing process or had just re-entered the lumen, have been attributed to secondary infections from the ingestion of unhatched eggs voided by the experimental chickens.

DISCUSSION

The results obtained on the mucosa-larvae lend support to the findings of Ackert (1931) who concluded that the tissue phase of this nematode lasted from about the 10th day to the 17th day. This period coincides closely with that of the heaviest mucosal infections found in this study (8th–17th day). Roberts (1937) found that *A. galli* larvae were in this phase from the 10th to the 19th day of parasitism. Early

in the present study, Ackert and Tugwell (1948) stated that the tissue phase may begin as early as the 3rd day and occur as late as the 23rd day of parasitism. Since then the results of experiments in this series have increased this period from the 1st to the 26th day after inoculation. This extends considerably the possible length of the tissue phase.

The growth rates of the lumen and mucosa-larvae, respectively, correspond very closely to the (Ackert, Whitlock and Freeman, 1940) normal growth rate of worms from the intestinal lumens of regularly fed chickens, and to the slow growth rate of young worms from the intestinal lumens of chickens fed only by intramuscular injections of glucose. This indicates that in the present studies little or no food was ingested by the mucosa larvae.

The failure of mucosa-larvae to grow after the first few days and the indication that they do not feed raises the question of their removal by the oral administration of anthelmintics to fowls. If these mucosa-larvae are not removed by anthelmintics, treatment must be repeated at intervals. To be most effective, treatment should be applied about two weeks after the peak of fowl illness due to *A. galli,* or at about monthly intervals during the first three months, when chickens are most susceptible to this intestinal ascarid.

SUMMARY

1. A study was made on 5 groups of chickens of about 25 each inoculated with infective eggs of the fowl nematode *Ascaridia galli* (Schrank) to determine details of the tissue phase of the life cycle of this parasite.

2. Larvae free in the intestinal lumen (lumen-larvae) were removed by flushing with warm water under pressure. Collection of larvae partially imbedded in the intestinal mucosa (tissue phase) was accomplished by application, apparently for the first time to a problem of this kind, of a mass digestion apparatus.

3. Whereas the tissue phase was thought to be from the 10th to the 17th day of parasitism, the present studies show that it may begin on the 1st day and that it may occur as late as the 26th day of parasitism.

4. The great majority of the young *Ascaridia,* however, appear to make their sojourn in the intestinal mucosa from about the 8th to the 17th day of parasitism.

5. No appreciable differences in the growth rates of the larvae recovered from the lumen and those isolated from the intestinal mucosa (mucosa-larvae) occurred until the 14th day, after which the lumen-worms appeared to grow at normal rates, while the mucosa-larvae showed little or no growth.

6. Of the four moults characteristic of this nematode, the 1st occurs in the egg, the 2nd moult of the lumen-larvae took place in the 6 to 8-day period, the 3rd during the 14th and 15th days, and the 4th moult occurred in the 18- to 22-day period.

7. Moulting of mucosa-larvae was irregularly retarded. Evidences of the 2nd moult occurred from the 8th to the 17th day; and completion of the 3rd moult was not noted in mucosa-larvae.

8. Failure of the 14- to 26-day mucosa-larvae of *A. galli* to grow at rates comparable to those of the lumen-larvae of these ages is attributed to retarded moulting of larvae in the tissue phase.

9. Mucosa-larvae appear not to be developed for effective feeding or major growth.

10. Diminutive larvae occurring among normal sized larvae in lumen flushings doubtless are mucosa-larvae which have been swept from their mucosal moorings in the flushing process, or have just re-entered the lumen.

11. Mucosa-larvae as well as lumen-larvae appeared to be somewhat restricted in numbers by increased natural resistance of chickens parasitized when 60 days old as compared with those from younger fowls of respective ages 23 and 38 days.

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SYLVATIC PLAGUE STUDIES

VIII. Notes on the Alimentary and Reproductive Tracts of Fleas, Made During Experimental Studies of Plague

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Much of the relatively simple internal anatomy of fleas is readily visible, particularly in lightly sclerotized species such as *Xenopsylla cheopis* (Rothschild, 1903), with the aid of a compound microscope using transmitted light and a low-power (×10) objective. The alimentary canal of fleas and the digestion of blood meals have been described in detail by Faasch (1935). In plague transmission experiments, progressive formation of plugs of plague organisms in the ventriculus and proventriculus of fleas has been photographed and studied (Eskey and Haas, 1940). The fleas were placed in a drop of water on a microscope slide before covering it with a cover slip. Peristaltic action of the ventriculus was temporarily interrupted by the immersion which stopped movement of the developing bacterial plugs sufficiently to allow the making of photomicrographs. In another study, fleas were retained on a slide without water through the gentle application of pressure on the restraining cover slip by one of the observer's fingers (Burroughs, 1947). The observations reported herein were made using Burroughs' technique and are part of experimental plague transmission studies described elsewhere (Holdenried, 1952). In particularly lightly sclerotized specimens, careful observation revealed even the heart beat in the dorsal portion of the abdomen. The pumping action of the pharyngeal pump, eggs in various stages of development, salivary glands, fat bodies, malpighian tubules, tracheae, muscles and other structures were also observed. Three species of fleas, *Diamanus montanus* (Baker, 1895), *Polygenis gwyni* (Fox, 1914) and *X. cheopis,* were observed during plague transmission studies at the Hooper Foundation.

GLOBULES AND BUBBLES IN THE VENTRICULUS

Frequently the ventriculus of *D. montanus* contained small, apparently clear globules within an hour after ingestion of a meal, but these usually were absent next day. Thirty-five had them after from 1 to 6 successive meals. Only twice were they found on days when the fleas had not fed. The globules were also observed in *X. cheopis,* but not in *P. gwyni.* Whether or not the globules observed in the present studies were fat was not determined.

In a group of unfed *P. gwyni,* which had emerged as adults up to 14 days previously, the ventriculi of the females contained from 1 to 6 large, clear bubbles, each of which seemed to dilate the organ. Immediately after the first blood meals, the fleas were re-examined and 29 of 45 females and 5 of 29 males still retained the bubbles and these filled about a fourth of the lumen of the ventriculus. They were eliminated rapidly and some fleas were free of them within an hour after feeding. Within 24 hours all had disappeared. Bubbles were not seen in *D. montanus* or *X. cheopis.* These may have been gas bubbles composed of carbon dioxide and

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oxygen similar to those found by Faasch (1935) in the ventriculi of fleas after long periods of starvation.

DARK PARTICLES IN THE VENTRICULUS AND PROVENTRICULUS

In the plague transmission experiments, fleas were examined both before and after feeding. After a plague-infected blood meal, residues appeared and dark particles formed in the alimentary canal. The particles persisted and were later churned about in fresh blood in the ventriculus by peristaltic action. Similar-appearing dark particles could also be seen in uninfected fleas, but they broke up and disappeared after fresh blood was ingested. Fleas without the persisting type of dark material in their proventriculi or ventriculi were usually plague-free on bacteriologic culture.

In one group of *D. montanus* fed on uninfected mice and examined microscopically at intervals for 3 weeks, blood residues cleared from the alimentary canal at varying rates. Normally after a blood meal, the swollen bright-red ventriculus gradually shrinks, and the contents become, within 24 hours, a duller red, then brown and at times even black. The proventriculus of fed fleas frequently becomes dark and remains so for several days, indicating that some of the blood residue remains in this region. In some *D. montanus,* all residue cleared completely within 24 hours. Others retained in the ventriculus a reticulate brownish material which disappeared gradually. Partially digested blood remained in the ventriculus of 2 normal fleas 7 days after the last feeding; in another flea, for 8 days, and in still another, for even 10 days after the last blood meal.

PROLONGED FUNCTIONING OF THE PHARYNGEAL PUMP

The pharyngeal pump of the flea is situated beneath the frons of the head capsule and normally it probably functions only during feeding, propelling blood posteriorly into the esophagus and ventriculus. In from 1 to 4 days preceding death of 12 *P. gwyni* in which the alimentary tract was plugged with plague bacilli, this pump worked strenuously after the fleas were removed from the mice on which they had been fed. In 6 instances this vigorous pumping was noted only on the day of death or the day preceding it; in 2, for 2 days prior to death; in 2 more, 3 days before death; in 1, more or less continuously for the last 4 days of life. The pumping, usually with blood in the anterior part of the esophagus, may have represented an effort to force a passage through the occluded proventriculus. The continued pharyngeal pumping after removal of the flea from the host was occasionally noted in other species in which the alimentary canal was occluded, but never with the vigor of *P. gwyni.* Perhaps in this robust species when the alimentary canal is plugged and the flea nears death, it retains sufficient energy and muscular co-ordination to maintain the pumping longer than do fleas of the other species studied.

DISPLACEMENT OF THE "BLOCKED" GUT

Plague-infected fleas, particularly those with digestive tracts obstructed with plague organisms, usually gradually shrink while they are still alive. The abdominal segments telescope anteriorly into each other, increasing the opaqueness of the abdomen until the internal abdominal organs can no longer be seen under the microscope. In both *X. cheopis* and *P. gwyni,* the alimentary canal and other abdominal organs are forced forward into the thorax since it is more rigid and does not con-

tract. The proventriculus moves anteriorly from the first abdominal segment and is forced into the area of the mesothorax, even occasionally into the pronotum. The esophagus, expanded with blood, is forced upward to the notum of the thorax; then, depending on the degree of compression, it bends ventrally into the sternal region. In 4 *P. gwyni,* the esophagus was forced into either the first or the second pleuron. A similar extreme displacement was noted in a *X. cheopis.* While the *D. montanus* nearing death frequently also looked desiccated and shrivelled, the esophagus never became quite as displaced as it was in the other two species, possibly because only a comparatively few fleas with occluded alimentary canals were observed. The shrunken fleas always died, usually within a day or two. Normal, uninfected fleas, especially those starved for long periods, shrivelled and died, but the position of their clear and transparent alimentary canals was not visible under the microscope.

EXPULSION OF FECES

Since fleas harbor *Pasteurella pestis* in the alimentary canal, the rate at which ingested blood appears in the feces and the amount expelled have been considered to be correlated with the rate at which infecting organisms are passed from the flea. The amount of feces and time after feeding that they were eliminated by the three species studied differed markedly. Males defecated smaller amounts than females, probably because the males ingested less blood. Within a few days after feeding, the bottoms of the vials containing individual *D. montanus* females became soiled with feces, while the vials of male *D. montanus* remained relatively clean. Female *D. montanus,* when pressed between a glass slide and cover slip for observation after feeding, usually expelled a sticky fluid. Other species did this only rarely. *P. gwyni* in vials defecated infrequently after the first few engorgements. Two groups of about 45 plague-infected *P. gwyni* each were fed collectively on muzzled white mice; in about 2 hours they defecated enough to discolor the mouse hair. Other *D. montanus* and *X. cheopis* fed at the same time produced noticeably smaller quantities of feces. After the first two feedings, however, the hairs of *P. gwyni* hosts were much less discolored; by the fourth feeding, feces were not seen in the 2 hour period. These fleas appeared to ingest more blood on the first feedings and passed large quantities of feces; as the number of feedings increased, the amount of blood ingested and feces passed decreased. No fleas squirted undigested blood from the anus while feeding. The fecal deposits were dark, indicating the evacuation of the remains of previous meals rather than of freshly ingested blood.

EGG PRODUCTION

Developing egg cells were observed in plague-infected females. Eggs developed to a size easily visible under the microscope 8 days after the first blood meal. Four to 6 large eggs were laid in a day, leaving the ovaries apparently empty, but a day later new small eggs could be seen. Field-collected *D. montanus* continued to produce eggs after obtaining plague-infected blood. Twelve successive batches of eggs were laid by one of these females. Another *D. montanus* with its alimentary canal plugged with *P. pestis,* causing regurgitation of ingested blood, produced eggs in spite of this interference with normal digestion.

A normal *D. montanus* contained eggs on 11 of 13 observations made over a 19 day period. The number visible at one time varied from 1 to 4. This observa-

tion indicates that this species is capable of producing eggs rather continuously for about 3 weeks.

X. cheopis with alimentary canals completely obstructed with plugs of *P. pestis* continued to produce eggs. One such female, unable to feed for 8 days because of such a plug, had 6 eggs in her ovaries. Another flea ingested blood containing plague organisms and was starved for 36 days. Then, after she fed on the 37th through the 40th day, 4 eggs developed in her ovaries. When she was killed 9 days later, 6 new eggs had developed and she was found on bacteriologic culture to have remained plague-infected.

The ovaries of *X. cheopis* continued to function after the anterior alimentary canal became so obstructed that blood could not be forced into the ventriculus. However, eggs were never observed in shrunken specimens, and it is doubtful that fleas reaching this stage can produce eggs.

SUMMARY

Live *D. montanus, P. gwyni* and *X. cheopis* were examined microscopically and the following observations were made: (1) Clear globules formed in the ventriculi of *D. montanus* and *X. cheopis,* but not of *P. gwyni.* Bubbles present in some starved *P. gwyni* are rapidly eliminated after feeding. (2) Persistence of dark particles in the ventriculus after ingestion of a fresh meal of blood containing *Past. pestis* indicate that the flea is plague-infected. Visible remains of previous blood meals in normal fleas may disappear in as little as 24 hours, but occasionally persist for as long as 10 days. (3) Continued action of the pharyngeal pump in fleas with plugged alimentary canals is most persistent and vigorous prior to death in *P. gwyni.* This also occurred in other species. (4) The abdomen of a flea with its digestive tract obstructed with a plug containing plague organisms may telescope anteriorly forcing the proventriculus into the pronotum, displacing the esophagus. (5) The amount and rate of feces expelled does not seem to affect the rate at which fleas become free of plague organisms. (6) Plague-infected fleas are capable of egg production.

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THE SURVIVAL OF *TOXOPLASMA GONDII* IN VARIOUS SUSPENDING MEDIA

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In the handling of *Toxoplasma gondii* outside its host organisms, for such purposes as the preparation of standard inocula for test animals, some assurance is necessary that the toxoplasmas remain viable and infective in the media used. If the medium is not adequate, no real standardization of inocula is possible, especially when the time which elapses following removal of the parasite from the host is extended. Because of these considerations, it has been our practice (Jacobs and Jones, 1950) to limit the time of handling to one hour. However, it is possible that even within such a short period deleterious effects on the toxoplasmas might result from an inadequate medium. Consequently it was considered necessary to assess various fluids for their ability to sustain toxoplasmas without harm and to ascertain reasonably safe time limits for the handling of the parasites *in vitro*.

Some data have been reported in regard to the length of time that *Toxoplasma*-infected tissue may be stored without loss of the parasite. Sabin and Olitzky (1937) stated that the parasites could be preserved by storing an infected mouse brain in Tyrode's solution in the refrigerator without loss of infectivity for 14 days. Wolf, Cowen, and Paige (1940) kept pieces of brain and spinal cord of a fatal neonatal case of toxoplasmosis in sterile physiological saline for 11 hours at 7 degrees C. without noting any essential difference from fresh material in subsequent inoculations into mice. Manwell and his collaborators (1945) stated that infected tissues kept in the refrigerator seldom showed living toxoplasmas after 48 hours. More recently, however, Manwell and Drobeck (1951) report survival of toxoplasmas in infected mouse brains for as long as 18 days when the tissues were suspended in Difco skim milk and kept in the refrigerator; they noted no differences in the time of development of infections in mice inoculated with stored material. To our knowledge, beyond statements on the susceptibility of toxoplasmas to freezing and drying, there has been no report on the survival of small numbers of the organisms in various fluids which might be used for handling *Toxoplasma* in the laboratory. In this paper will be presented data on the effects of tissue storage on the toxoplasmas and information on the survival of small numbers of the parasites in saline, broth, and other fluids.

MATERIALS AND METHODS

The RH strain of *Toxoplasma gondii* originally isolated by Sabin from a fatal neonatal case of human toxoplasmosis, and supplied to us by Dr. Isaac Ruchman, was used in all tests. The organism was maintained in mice by the intraperitoneal

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inoculation of peritoneal fluid or triturated tissues of previously infected mice, or by the intracerebral inoculation of 0.02 ml. of triturated brain. In the storage experiments, brains of intracerebrally infected mice were placed in neopeptone broth at 5 degrees C. and stored for 7 or more days. After this time, the tissue was triturated in saline sufficient to make a 10 percent suspension and inoculated intraperitoneally into 2 mice. If the recipient mice died of toxoplasmosis, the survival time was noted; in some cases survivors were sacrificed and their brains, livers, and spleens pooled, comminuted in saline, and inoculated intraperitoneally into 2 more mice. The data on the survival of toxoplasmas in various fluids were obtained by preparing suspensions of the organisms in saline, 10 percent serum-saline, and 1 percent neopeptone broth, and observing the ability of these suspensions, after standing for various periods, to produce infections on inoculation intraperitoneally into mice. The suspensions were prepared from peritoneal fluid in which the parasites were present mostly as single, free organisms rather than clumped within parasitized cells or the remains of cells, and were adjusted by dilution in the proper fluid so that 0.5 ml. was calculated to contain the desired number of organisms for inoculation. In order to prevent the nutrient fluids used from serving as substrates for bacterial growth, small quantities of penicillin and streptomycin, 50 units and 0.5 mg. per ml. respectively, were added to all the suspensions.

TABLE 1.—*The infectivity of* Toxoplasma-*infected mouse brain tissue after various periods of storage in neopeptone broth at 5° C.*

Storage period (days)	No. of specimens tested	Results: Number of specimens positive	Survival time of mice* (days)
7	3	3	8
8	1	1	6
10	2	2	8
12	1	1	8***
14	5	1	7
15	1	1	8
17	1	1	12**
21–32	6	0	..

* Two mice were inoculated with a 1 : 10 suspension of the tissue in the test of each specimen. Survival time noted is the average for the mice which died of toxoplasmosis.
** Only 1 mouse died.
*** S——Mice of the first inoculation survived. The infection was found in a second series of subinoculated mice.

RESULTS AND DISCUSSION

Table 1 presents the results of six experiments involving a total of 20 tests on the infectivity of stored *Toxoplasma*-infected brains. In all tests in which the tissues were stored for 10 days or less, inoculation of the material into mice resulted in *Toxoplasma* infections; in all but one instance, the infections were found in the first pair of inoculated mice. Even after 12 days storage, a few viable toxoplasmas were present in some of the tissues; this was evidenced by the production of infections in a second series of mice inoculated with tissues from the mice of the first test. Beyond 14 days storage, the results showed considerable variation. In only 1 of 5 tests of material stored for 2 weeks, was *Toxoplasma* found. In single tests made at 15 and 17 days storage, viable organisms were demonstrated. In the 17-day test, one of the two mice inoculated with the stored material died on the 12th day. This is a very extended survival time, considering that inoculation of a similar quantity of fresh brain practically uniformly produced death in 4 days. Considering

this normal survival period of mice infected with fresh tissue, it was apparent that even after storage for 7 days there was a considerable drop in the number of infective organisms in the tissues, as evidenced generally by the doubling of the survival time of the mice. Hence it is believed that storage adversely affects the toxoplasmas in tissue and that the continued infectivity of stored material is due to the fact that of the tremendous numbers of organisms originally present in infected brains, relatively small numbers remain viable. Without knowledge of the numbers before and after storage it is not possible to assess the rate of loss of viability of the parasites. It was noted also that among the various experiments there were 3 particularly successful ones, in which more infections were obtained or longer periods of infectivity of the tissues were demonstrated. In the other experiments, survival of the toxoplasmas was demonstrated after storage for 10 days in 1 case and after 7 days in the other 2 instances. These latter were not tested at 10 days, but were negative at 14. It is considered likely that in the particularly successful tests, the brains were more heavily parasitized than in the other experiments, and consequently remained infective longer even though attrition may have taken place at

TABLE 2.—*Survival of toxoplasmas in various fluids as determined by mortality of mice inoculated with such suspensions after designated periods of standing.*

Suspending Fluid	Number of toxoplasmas inoc.	Storage temp. (°C.)	Number of mice dead/Number inoculated with designated toxoplasma suspensions kept for					
			1 hour	2–3 hours	4–5 hours	7–9 hours	13 hours	24 hours
Saline	10	20–28	15/16(9.5)*	2/16(9.5)	0/16			
	10	5	....	2/8(10.5)	0/8			
	50	20–28	13/16(9.6)	7/16(10)	0/16			
	50	5	.....	3/8(9.7)	1/8(10)			
	100	20–28	4/4(8)	4/4(10.3)	0/4			
	100	5	.....	4/4(9.3)	1/4(9)			
1 percent neopeptone broth	10	20–28	11/12(9.8)	8/8(9.1)	8/8(9.5)	3/8(9)		
	50	20–28	12/12(8.5)	8/8(9)	8/8(9)	3/8(9.3)		
10 percent** serum-saline	10	20–28	4/4(9)	....	4/4(9.5)	....	2/4(8.5)	3/4(10.3)
	50	20–28	4/4(8.5)	....	4/4(8.5)	....	4/4(9.7)	4/4(10)

* Numbers in parentheses give the average survival time in days of the mice which died of the *Toxoplasma* infection.
** Normal human serum was used in all tests with serum.

the same rate. On the basis of the results reported here, for practical purposes 7 days might better be considered the maximum period of storage at 5° C. without complete loss of viability of the toxoplasmas in infected tissue.

In order to obtain a better idea of the ability of various fluids to sustain *Toxoplasmas gondii in vitro,* it was decided to perform tests with definite, small numbers of parasites over a shorter period of time. The general method of performing these tests has already been stated. The preparation of the various suspensions of toxoplasmas was accomplished within 1 hour after the peritoneal fluid was withdrawn from the mouse. At the end of this first hour, groups of 4 mice each were inoculated with 0.5-ml. aliquots of the suspensions. At various later intervals, additional inoculations of mice were made.

In table 2, the results of a number of experiments are combined to illustrate the poor survival of *T. gondii* in saline. Even after 2 hours at room temperature, an inoculum of toxoplasmas suspended in saline had lost considerable ability to produce infections in mice. Thus, 15 of 16 mice died following inoculation with suspensions containing 10 toxoplasmas per 0.5 ml. when the inoculation was performed

1 hour after removal of the parasites from the host. Only 2 of 16 mice died after inoculation with the same suspensions 1 or 2 hours later. A similar decrease in numbers of infected mice is noted with the larger inocula, except in the case of an inoculum of 100 organisms. Here, however, it can be seen that the survival time of mice infected with the suspension after it had stood for 2 or 3 hours was increased almost 2.5 days, which indicates that the infections probably resulted from much fewer than the original 100 parasites. After 4 to 5 hours standing at room temperature, the 100-toxoplasma suspensions failed to produce any deaths in mice inoculated with them. A hardly better survival of toxoplasmas occurred in the refrigerated saline suspensions.

In table 2, there are also presented the results of similar experiments with 1 percent neopeptone broth in saline and with 10 percent serum-saline. It is obvious that the additional ingredients in the suspending medium make it considerably superior to saline. Even after 4 or 5 hours, suspensions of toxoplasmas in broth or serum-saline remained 100 percent infective to mice, and there was no appreciable lengthening of the survival time of mice inoculated with them. After 7 hours, some decrease in survival of toxoplasmas in these fluids could be noted, but 3 of 4 mice died following inoculation with 10 toxoplasmas kept at room temperature for 24 hours in serum-saline. Results with the same suspensions kept at 5° C. were similar.

In table 3 are presented the results of 2 experiments in which saline and 10 percent serum-saline were compared on the same material in parallel tests. The

TABLE 3.—*Comparative survival of toxoplasmas in saline and in 10 percent serum-saline as indicated by the mortality of mice inoculated with such suspensions after various periods of standing*

	No. of toxoplasmas inoc.	Suspending Fluid	Temp. (°C.)	No. of mice dead out of 4 inoculated with toxoplasma suspensions kept for			
				1 hour	5 hours	13 hours	24 hours
Exp. A	20	Saline	20–27	3(11)*	0	0	.....
	20	10% Ser.-Sal.	20–27	4(9.8)	4(9.8)	2(12.5)	.....
	20	Saline	5	.....	0	0	0
	20	10% Ser.-Sal.	5	.....	4(10)	2(9.5)	1(10)
Exp. B	10	Saline	20–27	4(8.1)	0	0	0
	10	10% Ser.-Sal.	20–27	4(7.1)	4(8)	4(10)	3(10)
	10	Saline	5	.....	0	0	0
	10	10% Ser.-Sal.	5	.....	4(8.1)	4(8.1)	4(9.1)
	50	Saline	20–27	4(7.1)	0	0	0
	50	10% Ser.-Sal.	20–27	4(7.1)	4(7.5)	4(8)	4(10)
	50	Saline	5	.....	0	0	0
	50	10% Ser.-Sal.	5	.....	4(7.7)	4(8)	4(8.8)

* Figures in parentheses give the average survival time in days of the mice which died of the *Toxoplasma* infection.

advantage of the latter suspending fluid is again obvious. In experiment A, an inoculum of 20 toxoplasmas in serum-saline was still capable of producing death in all 4 mice after it had stood for 5 hours at either room or refrigerator temperatures, whereas the saline suspensions were not infective after that period. Experiment B shows an even better survival of the parasites in the serum-saline. An inoculum of 10 toxoplasmas in serum-saline was capable of killing all 4 mice even after standing 24 hours at 5 degrees C.

The results of similar experiments comparing saline with broth and milk indicate that these fluids are also superior to saline. Some survival of toxoplasmas in broth and milk was found after 9 hours, in tests in which the parallel saline sus-

pensions showed considerable loss of viability at 1 hour. The results are therefore comparable to those of experiment A. Since in these tests the toxoplasmas are kept in saline until the various dilutions are prepared, it is probable that loss of viability in the saline may adversely affect the results obtained with the other media.

There can be no doubt that saline itself is a very unsatisfactory medium for maintaining toxoplasmas *in vitro* for even very short periods of time. Even under the best of circumstances, survival of the parasites cannot be expected for more than 2 or 3 hours, and in some instances considerable loss of viability can be found within 1 hour. The variation in saline suspensions may be explained on the basis of variability in the protein content of the peritoneal exudates which serve as the source of toxoplasmas for the tests. If one exudate is considerably richer in protein than another, the toxoplasmas may suffer less loss of viability in it. This point may have considerable significance for workers who use the survival time of mice inoculated with MLD_{50} dilutions of the toxoplasmas as a criterion for differentiating strains of *Toxoplasma gondii* on the basis of virulence.

The addition of broth or normal serum results in lessening the adverse effects of saline on toxoplasmas *in vitro*. Even in the presence of serum or broth, however, survival of all the parasites in a suspension cannot be assured for longer than a few hours. It is our practice, now, in preparing inocula, to make all the necessary dilutions in serum-saline and to utilize the material as rapidly as possible. Serum-saline has also been useful to us in mailing infected tissues from one laboratory to another. Thus far we have been successful 3 of 4 times in establishing *Toxoplasma* strains in mice following air mail shipments of infected tissue with a time lapse of 24 to 48 hours.

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CECAL COCCIDIOSIS AND CARBOHYDRATE METABOLISM IN CHICKENS*

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INTRODUCTION

In recent years a certain amount of interest has been shown in problems relating cecal coccidial infections in chickens with the physiological conditions produced in the host; Herrick, Ott, and Holmes (1936), Pratt (1940, 1941), and Waxler and Herrick (1941). In a renewal of these investigations attention is being directed toward the metabolism of the host as an index of the relationship between host and parasite.

The need for more information concerning the physiological host-parasite relationship of *Eimeria tenella* and the domestic fowl is indicated by a marked inadequacy of any present theory in explaining certain outspoken manifestations of the infection. Pratt (1941) attempted to demonstrate that the hemorrhage which accompanies severe infections was the basic cause of the upset physiology in the chicken, but his experiments were not conclusive. The present work is directed toward a study of the influence of acute coccidial infections on the carbohydrate metabolism of the host.

EXPERIMENTAL

Preparation of tissue. Pure bred Single Comb White Leghorn chickens were used in all the experiments. These chickens were all from the same parental stock and were bred by artificial insemination. They were genetically bred for susceptibility to coccidiosis. The eggs were incubated by the Department of Poultry Husbandry of the University of Wisconsin and the chicks were delivered when one day old. They were kept free from contamination with coccidiosis until infected experimentally. Their age at this time varied from four to eight weeks. For infection, 100,000–150,000 sporulated oocysts were given orally with a one milliliter tuberculin syringe. This was found to induce a mortality rate of from twenty to thirty per cent. The oocysts used in producing the infection were prepared from previously infected chickens.

In preparing the tissues for study homogenates in part were used. The animals were decapitated, the desired tissue quickly excised, weighed and immediately homogenized in cold glass homogenizing tubes with sufficient water to make a dilute paste. Water was then added in the proper amounts to give the desired per cent homogenate. The R.Q.'s were calculated after Umbreit, Burris and Stauffer (1945). Chicken brain tissue was chosen for the assays of carbohydrate metabolism

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since, as will be seen below, it is capable of metabolizing both glucose and HDP substrates.

Experimental procedure. Assays of carbohydrate metabolism were made manometrically in Warburg flasks at 38° C. For these studies the medium in the flask was adapted from that reported for use in studying the anaerobic glycolysis of tumor by Novikoff, *et al* (1948). Initial tests were made to insure the presence of each constituent in its optimum amount. The final medium was composed of the following materials: 0.3 ml., 0.1 M K_2HPO_4/KH_2PO_4 buffer of pH 7.6; 0.3 ml., 0.5 M $KHCO_3$; 0.1 ml., 0.01 M adenosine-tri-phosphate (ATP, potassium salt); 0.3 ml., 0.4 M nicotinamide; 0.1 ml., 0.5% diphospho-pyridine-nucleotide (DPN, potassium salt) in side arm; 0.1 or 0.5 ml., 0.4 M hexose-diphosphate (HDP, potassium salt), depending on the experimental conditions; 0.1 or 0.5 ml., 0.28 M glucose, according to the desired conditions; 0.1 ml., 0.1 M $MgCl_2$; 0.1 ml., 0.015 M potassium pyruvate; and sufficient glass distilled water to make a final flask volume of 3.0 ml. on addition of the tissue homogenates. In these studies 0.3 ml. of a 5% homogenate of brain and 0.3 ml. of a 10% homogenate of cecal mucosa were used wherever these tissues were included in the reaction mixture. The HDP and glucose were varied inversely with each other in order to study the tissue's capacity to phosphorylate. It was not found advisable to completely eliminate the HDP or the glucose. The term glucose substrate will refer to a reaction mixture in which 0.5 ml. of glucose and 0.1 ml. HDP were included. A HDP substrate will refer to one in which 0.1 ml. glucose and 0.5 ml. HDP were included.

The experiments were evaluated primarily on the basis of carbon dioxide evolution from the bicarbonate buffer. Earlier work in this laboratory (unpublished data) involving measurements of lactic acid and inorganic phosphate esterification along with CO_2 had indicated that the increased CO_2 tension represented a reliable criterion for the estimation. The results are expressed in $Q_{CO_2}^{N_2}$ values.

Source and preparation of materials. The ATP was prepared as the barium salt after the method of Dubois, Albaum, and Potter (1943). For use in the flasks this stock material was converted into the potassium salt by dissolving 49.9 mgm. BaATP in 2 N HCl, adding 50 mgm. K_2SO_4 to precipitate the barium and centrifuging out the precipitate. The precipitate was washed with a small amount of water and again centrifuged. This supernatant was added to the first. The combined supernatants were then neutralized to pH 7.4 with cold 2 N KOH and made up to 5 ml. Fresh KATP was made up weekly by this process.

The DPN used in these experiments was obtained from Schwarz Laboratories, Inc. as the barium salt. Fresh potassium salts were made up weekly by dissolving 25 mgm. in 2.5 ml. water. To this was added 2.5 ml. 0.5% K_2CO_3 and the whole was aerated to remove the excess CO_2.

The pyruvic acid was obtained from the Eastman Kodak Company. It was redistilled under low vacuum, titrated and made to 1.0 N. Fresh potassium pyruvate for the flasks was made daily by diluting .75 ml. of 1 N pyruvic acid to 25 ml. with water and mixing it with an equal volume of 0.15 M K_2CO_3. The excess CO_2 was aerated off.

The HDP was obtained from Schwarz Laboratories, Inc. The flask solution was made up weekly by dissolving 408 mgm. BaHDP in 2 N HCl, adding 408 mgm. K_2SO_4 to precipitate the barium. This was neutralized with 2 N KOH. The neutralized KHDP was then made up to 15 ml.

RESULTS

Anaerobic glycolysis of brain. Whole homogenates of the brains of normal and infected chickens were studied to determine their phosphorylative capacities *in vitro*. The results are presented in Fig. 1. Little difference will be noticed on the two substrates and since the activity of brain homogenates from uninfected and infected chickens were indistinguishable they are graphed together. The slightly greater activity on the glucose substrate agrees with the known preference of mammalian brain tissue for this metabolic precursor.

Anaerobic glycolysis of cecal homogenates. The $Q_{CO_2}^{N_2}$ values obtained from studies of uninfected and infected cecal musoca homogenates are given in Fig. 2.

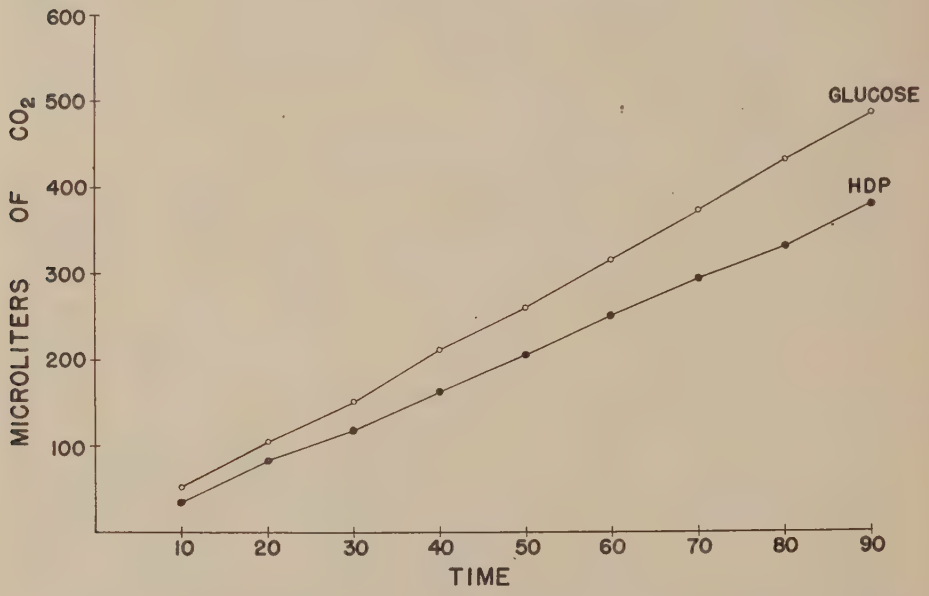

FIG. 1. CO_2 production by chicken brain on glucose and HDP substrates. The points from which these curves were constructed were based on the results of a minimum of twenty experiments run in duplicate on separate animals.

In both cases the homogenates were more active on the HDP substrate indicating a lack of ability to effectively phosphorylate the glucose *in vitro*. That this is not unusual was disclosed by earlier unpublished work in this laboratory; of all the tissues from the domestic fowl only brain tissue was capable of phosphorylating glucose *in vitro* under the conditions of these experiments. The lower values obtained with infected cecal mucosa are probably due to the inclusion of inert material (blood clots, etc.) with the tissue from which the homogenates were made.

Anaerobic Glycolysis of Normal Chicken Brain as Influenced by Uninfected an Infected Cecal Mucosa Homogenates. The results of these experiments demonstrate that a marked decrease in the phosphorylative ability of brain homogenates may be induced by the addition of infected cecal mucosa homogenates to the incubation flask (Fig. 3). This is evidenced by the sharp decline in the activity of the combined homogenates on a glucose substrate.

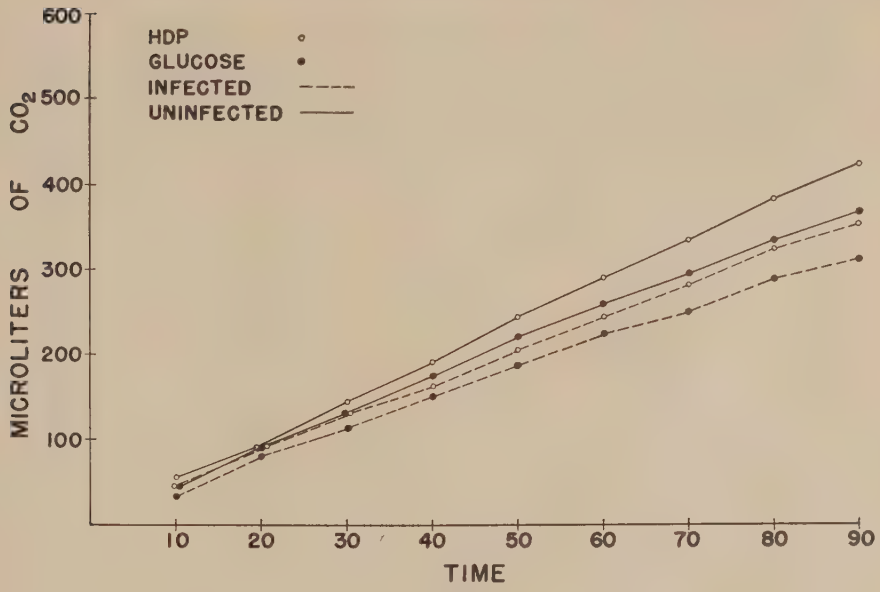

FIG. 2. CO_2 production by infected and uninfected chicken cecal mucosa on glucose and HDP substrates. The points from which these curves were constructed were based on the results of a minimum of twenty experiments run in duplicate on separate animals.

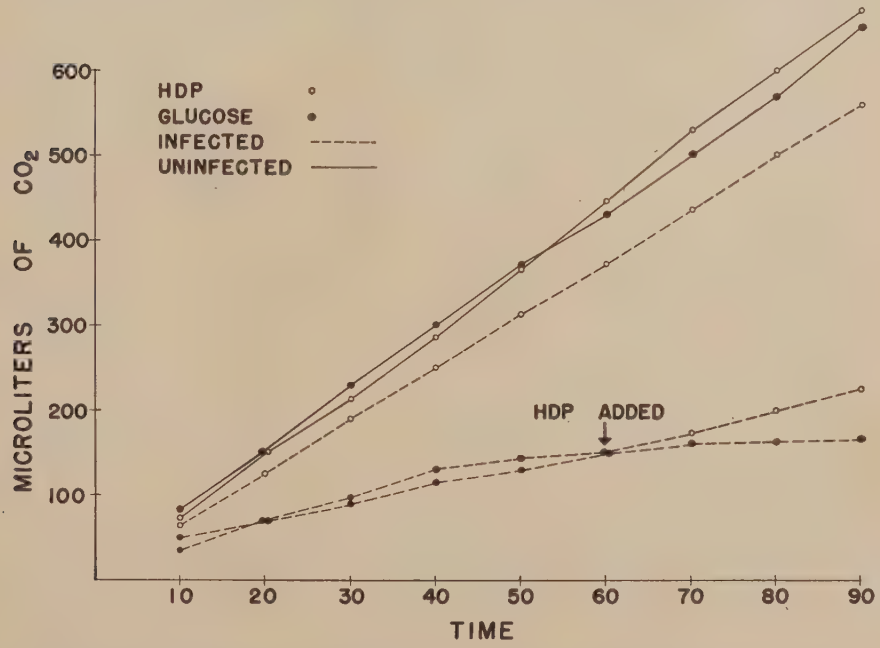

FIG. 3. CO_2 production by chicken brain with infected and uninfected cecal homogenates added on glucose and HDP substrates. The points from which these curves were constructed were based on the results of a minimum of twenty experiments run in duplicate on separate animals.

In the presence of uninfected cecal homogenates the brain tissue was uninhibited and the $Q_{CO_2}^{N_2}$ values represent the combined activity of mucosal and brain homogenates operating in the same reaction mixture. The degree of inhibition caused by the addition of the infected cecal homogenates varied widely from almost immediate cessation of CO_2 production to a more slowly developed effect. Table 1 gives

TABLE 1.—*Average* $Q_{CO_2}^{N_2}$ *values for chicken brain incubated with infected mucosa*

Time	Glucose Substrate	S.E.	HDP Substrate	S.E.
10	48.2(20.4–65.4)	± 2.42	63.2(55.1–69.7)	± .93
20	25.3(15.9–43.9)	± 1.71	62.3(54.9–70.0)	± 1.01
30	23.1(10.1–37.7)	± 1.91	63.5(56.0–71.0)	± .94
40	22.2(7.8–31.0)	± 1.72	65.4(51.7–69.5)	± 1.01
50	18.4(3.0–26.3)	± 1.63	63.1(53.5–70.4)	± 1.04
60	13.7(2.4–19.7)	± 1.44	60.1(55.5–71.1)	± 1.71
70	9.1(0.0–20.6)	± 1.04	61.4(57.4–68.9)	± 1.43
80	4.4(0.0–19.0)	± 1.01	62.8(54.3–65.8)	± 1.80
90	3.7(0.0–18.5)	± .98	58.7(50.1–64.4)	± 1.49

the spread of the $Q_{CO_2}^{N_2}$ values for 10 minute periods. In most cases the inhibition was complete by the end of 60 minutes, whereas the homogenates on an HDP substrate or a combination of brain and uninfected cecal homogenates on either substrate maintained a high degree of activity for two to three hours.

Figures in parentheses give the spread of the Q values from which the averages were taken. In two instances there was complete failure to obtain inhibition on the glucose substrate with brain and infected mucosal homogenates.

The addition of HDP (Fig. 3) at 60 minutes allowed a limited recovery from the inhibition obtained on the glucose substrate. This may be considered further evidence that the phosphorylation of glucose was involved in the inhibition.

Respiratory quotients of infected chicken muscle. In Table 2 are given the

TABLE 2.—*R.Q. determinations on muscle of normal, infected, and starved chickens*

Uninfected muscle	Infected muscle 5th day	Infected muscle 6th day	36 hours starvation	48 hours starvation	72 hours starvation
.89	.69	.68	.82	.86	.84
.93	.71	.67	.91	.89	.88
.91	.69	.73	.78	.92	.89
.84	.65	.75	.92	.87	.83
.86	.73	.71	.91	.85	.86
.88	.70	.67			
.90	.69	.59			
.99	.68	.62			
.89	.74	.69			
.87	.79	.73			
	.78	.70			
	.69	.75			
	.70	.71			
	.59				
Ave. .89	.70	.69	.86	.87	.86

results of several determinations of R.Q. values on uninfected, infected, and starved chicken muscle tissue.

From an average value of .89 with normal muscle the R.Q.'s dropped to an average of .70 on the fifth day after infection and .69 on the sixth day. Starvation failed to reduce the R.Q. of normal chickens.

DISCUSSION

Pratt (1941) postulated that the hemorrhage accompanying acute cecal infections of *Eimeria tenella* occasioned many of the physiological abnormalities ex-

hibited by infected chickens. These include: (1) increased blood sugar level, (2) lowered muscle glycogen, (3) and reduced blood volume. The conclusions he drew from artificial bleeding experiments are open to criticism. The elevated blood sugar levels he obtained by bleeding lasted for only a day or two, whereas the levels produced by infections persist much longer. He failed to obtain convincing evidence of glycogen reduction following bleeding, whereas it is easily demonstrated following infection. His technique involved the withdrawal of large amounts of blood in a rather short period of time. During an infection the over-all loss may be great, but it occurs by periodic losses of smaller amounts—too small in themselves to produce marked changes in carbohydrate levels.

Other conditions extant at the time of the high blood sugar levels, such as the low body temperature, muscular asthenia, the loss of ability to maintain body temperature in face of stress additional to the disease, and the lowered R.Q. values not produced by starvation, engender doubt that hemorrhage alone is involved.

All of these physiological conditions may conceivably be associated with the metabolism and distribution of carbohydrates in the body of the chicken. Conversely, a decrease in the ability of the tissues to metabolize carbohydrates would be reflected in a physiological pattern consistent with that shown during a severe coccidial infection. The data in Fig. 1 showing that the *in vitro* phosphorylation by brain homogenates was not inhibited in infected chickens is not considered an important objection to such a postulate as it must be remembered that these homogenates were operating under optimum conditions in a fairly well isolated system with no interference from or competition with other systems. Most of the essential materials were present in excess and any inhibitory material that might have been in the original tissue had been diluted markedly in preparing the homogenates. It is obvious at this time that there is a need to demonstrate an interference with carbohydrate metabolism in the body of an infected fowl. The R.Q. values given above hint at such a situation, although the recent work on carbohydrate synthesis and CO_2 fixation (Kalinsky and Werkman, 1944; Ochoa. 1948) has limited the conclusions that may be drawn from such figures.

Finally, as this work indicates, the presence of a material, as yet unidentified, in the cecum of infected chickens that is capable of stopping the phosphorylative mechanism must be added to the above considerations as evidence that the basic metabolism of the infected chicken is influenced in such a manner that phosphorylative carbohydrate metabolism is reduced. However, it must be recognized that these considerations are fundamentally presumptive and that further work dealing with the physiology of the infected chicken must be done before sufficient facts will have been obtained to allow for a full understanding of the nature and scope of the cecal coccidiosis.

SUMMARY

1. There is present in the cecum during the acute stage of the infection a substance capable of reducing the capacity of chicken brain to utilize glucose. This substance does not affect the utilization of hexose-diphosphate under the same conditions.

2. The R.Q.'s of muscle from infected chickens is reduced.

3. It is suggested that the physiological events associated with an infection of

cecal coccidiosis in chickens are induced by an interference with normal phosphorylative carbohydrate utilization.

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AN ANOMALOUS TAPEWORM FROM MAN

K. L. Hussey,[1] H. B. Shookhoff[2] and M. M. Sterman[2]

A very abnormal tapeworm was obtained from a patient at the Tropical Disease Diagnostic Service of the New York City Health Department.

I. *Case history and clinical findings.* The patient was a 39 year old housewife who was born in Mexico and lived there until May, 1947. She was first examined on November 9, 1949, and at that time gave a history of having passed portions of a tapeworm repeatedly over a period of about fifteen years. The pieces of worm were at first passed infrequently, approximately every two to three years. In the last three years, however, they had appeared every three to six months. The pieces of worm first appeared following a period in which the patient had been eating beef in an almost raw state. This had been prescribed because she had been found very anemic. The meat was prepared by merely searing it over a fire. She was treated only once for this tapeworm. That was in 1945 in Mexico when she was given a medication which she believes was male fern. Despite this treatment she continued to pass pieces of worm.

Physical examination was negative. Examinations of the stools revealed no eggs or other parasitic elements. She was told to bring in a portion of the worm the next time she passed it. On February 4, 1950, she brought in about 27 inches of the worm (Fig. 1). On March 8, 1950, she was given 0.8 gram of atabrine on an empty stomach and one hour later one ounce of 50% magnesium sulfate solution. Following this, she passed additional lengths of worm (Fig. 3) but no scolex was obtained. By comparison of size of the ends of the pieces, it appeared that they were parts of the same worm.

A blood count was done March 8, 1950. The results were as follows: hemoglobin 15.2 gm. per 100 cc.; red blood cells 4,900,000 per cu. mm.; white blood cells 10,850 per cu. mm.; neutrophiles 55%, eosinophiles 2%, lymphocytes 36%, monocytes 7%. Intradermal tests with *Dipylidium caninum* antigen and antigen derived from human echinococcus-cyst-fluid were strongly positive. *Fasciola hepatica* antigen injected intradermally gave a slightly positive reaction and *Trichinella spiralis* antigen gave no reaction. Complement fixation reaction with *Echinococcus* antigen was positive.

II. *Morphology.* The most striking feature of this worm is the complete lack of external segmentation and genital pores, and the presence of numerous papilla-like structures. These "papillae" are irregularly arranged close to both lateral margins and on both surfaces. Except for the most anterior portion obtained, they are found throughout the whole length of the worm. At any given location, they appear to occur in corresponding positions on both dorsal and ventral surfaces. Each papilla appears as an oval raised area with a more or less central elongate depression (Fig. 2), with no apparent connection with any internal structure.

From stained whole mounts and sections, it is possible to determine more of

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Fig. 1

Fig. 3

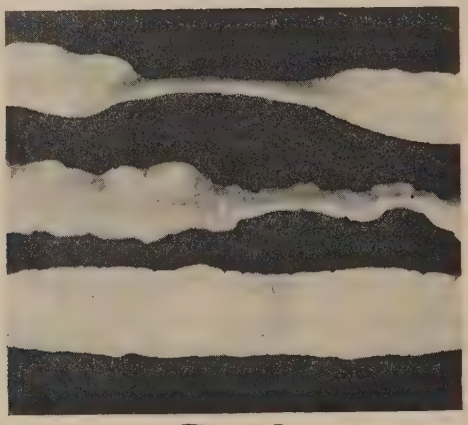

Fig. 2

FIG. 1. Piece 1; brought in on February 4, 1950.
FIG. 2. Detail of piece 1.
FIG. 3. Piece 2; passed on March 8, 1950, after treatment.

the anatomy, as follows: (1) there is no evidence of internal segmentation; (2) there is a prominent and dilated pair of lateral excretory trunks but no cross commissures; and (3) the most anterior portion (lower left portion in Fig. 3) shows well developed testes, but no other structures of the reproductive systems. Pieces taken further down, in the region of the "papillae", show what appear to be the main characteristics of the major part of the worm.

Male system—well-developed testes, vasa efferentia which appear to join and form the beginning of a vas deferens; in a few regions, a coiling of the vas deferens and the rudiments of a cirrus pouch near the lateral excretory trunk; no external opening to the system. Spermatozoa are present and pack the efferent ducts, causing considerable dilation, as would be expected with the lack of external opening.

Female system—uterine branches in a very irregular arrangement and with the characteristically rounded, finger-like branched tips, but empty of eggs. Uterine branches in some localities contain scattered bits of material that appear to be shell or vitelline material. Sections show a few groups of vitelline cells, but no trace of ovarian tissue. This accounts for the fact that there is no record of eggs being passed in the feces.

From this description, the worm appears to be a *Taenia,* but it is impossible to determine the species. Portions of this worm (whole mounts and sections) have been submitted to Dr. George R. LaRue of the Zoology Department of the University of Michigan for identification. He states that the specimen is undoubtedly a *Taenia.*

III. *Discussion.* The serological reactions also appear to confirm the diagnosis of *Taenia,* while the history of eating almost raw beef would give presumptive evidence of it being *T. saginata.* We have previously carried out intradermal tests with *Echinococcus* antigen in 11 cases of *T. saginata* infection. Six of the 11 showed positive reactions, three were doubtful and two were negative.

Brumpt (1922) described anomalies of *T. saginata* which include partial lack of segmentation, fenestration, bifurcations, etc. However, in his figure showing partial lack of segmentation, the irregularly alternate marginal genital pores, which are clearly shown, indicate the fundamental segmentation of the worm. In our search of the literature we failed to find either figures or descriptions of a type of anomaly similar to our specimen.

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CONTRIBUTIONS TO THE MORPHOLOGY AND LIFE-HISTORY OF *GYNAECOTYLA ADUNCA* (LINTON, 1905) (TREMATODA: MICROPHALLIDAE)

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Study of certain metacercariae found encysted in the fiddler crab, *Uca pugilator* (S. I. Smith), at the Duke Marine Laboratory at Beaufort, N. C., resulted in the tentative identification of these larvae as *Gynaecotyla adunca* (Linton, 1905). As a result of feeding experiments carried out during the summers of 1949 and 1950, adult worms were obtained which proved the identification and allowed a more complete description of the adult. Linton first described this form as *Distomum aduncum,* and he (1928) placed it in the genus *Levinseniella* (Stiles and Hassall, 1901). Rankin (1939) allocated it to his genus *Cornucopula;* in the same year Yamaguti included it in his genus *Gynaecotyla;* Rankin (1940) reduced *Cornucopula* to synonymy with *Gynaecotyla.* The genus belongs to the family MICROPHALLIDAE as emended by Cable and Kuns (1951). The species, therefore, has since been recognized as *Gynaecotyla adunca.* This paper deals primarily with feeding experiments and a redescription of the adult.

FEEDING EXPERIMENTS

Nestling birds were taken from local breeding areas near the laboratory. Seven young black skimmers, *Rynchops nigra nigra* Linn., twelve young common terns, *Sterna hirundo hirundo* Linn., and seven young least terns, *S. albifrons antillarum* (Lesson) were used. The *Uca pugilator* from which the metacercariae were taken were from one restricted area.

Metacercariae were removed from the fiddler crab and fed to the experimental birds. During the first 24 hour period after feeding the encysted metacercariae mature specimens of *G. adunca* were recovered from these hosts.

An analysis of the data in Table 1 shows a tendency towards an inverse correlation between the number of worms recovered and the elapsed time before autopsy of the hosts. The infection in the least terns decreased nearly 57 percent at the end of the first day. The drop in percentage of infection in the black skimmers was greatest after the second day. In the common terns, however, the percentage dropped more gradually. With so few hosts involved, and with the exception apparent in Table 1, it is suggested that the common tern is definitely the best of the experimental hosts and the least tern the poorest. Data obtained from routine examinations suggest that none of these birds is a natural host for *G. adunca.* Three adult black skimmers, three common terns, and one least tern autopsied in routine collecting yielded no worms of this species. No reference to them as hosts for this parasite has been found in the literature. In view of the fact that they maintained

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the worms for brief periods in the experimental work, however, it is assumed that they could carry the infection under natural conditions. Natural infections of *G. adunca* were found in the following birds: herring gulls, *Larus argentatus smithsonianus* Coues; Wilson's plover, *Charadrius wilsonia wilsonia* Ord; seaside sparrows, *Ammospiza maritima macgillivraii* (Audubon); and laughing gulls, *Larus atricilla* Linn.

Table 2 cites the natural definitive hosts which have been reported for *G. adunca*. In connection with these data, it is interesting to note that the original description of the adult worm was based on a specimen taken from the toadfish, *Opsanus tau* (Linn.). Measurements of eggs given in the original description

TABLE 1.—*Summary of feeding experiments*[a]

Expt'l Host	Number of cysts fed	Number of hours of development in host	Number of adult worms recovered	Percentage of infection
Black skimmer (*Rynchops nigra nigra*)				
1	50	26	16 (plus 4 unopened cysts)	32
2	81	36 (approx.)	14 (plus 2 unopened cysts)	17.3
3	72	48	24	33.3
4	72	96	none	
Common tern (*Sterno hirundo hirundo*)				
1	50	27	28	56
2	110	40	27	24.5[b]
3	100	64.5	47	47
4	100	88.9	34	34
5	75	120	none	
6	95	121	none	
7	75	136	none	
8	75	140	18	24
9	97	145	11	11.3
10	74	166	none	
11	91	193	none	
12	83	217	none	
Least tern (*S. albifrons antillarum*)				
1	50–60	15½– 21	33	60
2	"	22– 28	2	3.6
3	"	85– 93	3	5.4
4	"	90– 98	3	5.4
5	"	111–117	1	1.8
6	"	113–119	2	3.6
7	"	113–119	none	

[a] A total of 5 control birds was used in the experiments with the black skimmers and common terns. All of these were negative for *G. adunca*. Strigeids were commonly present in all but six of the birds used. Two acanthocephala were found, one in a skimmer and the other in a least tern. Cestodes were present in 2 skimmers. No controls were used with the least terns because the birds were taken from the nests upon hatching.

[b] Bird sick. Refused food 12–15 hours before autopsy.

[c] 55 was used as an average of the number of worms fed. With the exception of a few worms recovered from the first least tern, all of the *Gynaecotyla* were taken from the rectum of this experimental host.

imply maturity. Five of 136 toadfish were reported by Linton (1905) as being infected. Subsequent authors assumed that the toadfish was an accidental host (Rankin, 1939). Nine of 25 toadfish examined in this laboratory contained *G. adunca;* less than one-half of the worms were considered to be mature. Two of six puffers, *Sphaeroides maculatus* (Block and Schneider), were found to be carrying immature specimens. Hopkins (1940) reported several of these worms from four trigger fish, *Balistes carolinensis* Gmelin; none of his specimens contained eggs. Only three trigger fish have been examined by the writer, and none carried the parasite in question. One does not usually expect to find both fish and birds acting as definitive hosts for the same parasite; it therefore seems logical to assume that any fish containing these worms are accidental hosts. Of the reported hosts, the herring gull is the only one recorded as having a heavy natural infection of mature worms.

Data from these feeding experiments and from *in vitro* culture experiments (Hunter and Chait, 1952) support the opinion that many microphallids lack host-specificity (Rausch, 1947; Cable and Kuns, 1951). It is suggested, furthermore, that *G. adunca* lives but a short period as an adult. The rapid maturing of the worms, evidenced by the fact that eggs appear and are shed within an exceedingly short time (Hunter and Chait, 1952), may well be correlated with the wide variety of hosts reported for this species. Elucidation of the complete life cycle and developmental data will no doubt aid in answering these questions.

TABLE 2.—*Host list for* Gynaecotyla adunca *(Linton, 1905)*
(Experimental hosts are not included.)

Host	No. Examined	No. Infected	Date of examination	Reported by	Comments
Birds					
Crocethia alba	1	1	August 1902	Linton 1928	*Levinseniella aduncum.* Single specimen, #7941 U.S.N.Mus. Identical with form in toadfish?
Calidris arenaria (synonym of *C. alba*?)	1	1	August 1902	Linton 1905	"Apparently identical" worm as described from toadfish.
Larus atricilla	3	1	August 1948	This paper	Many mature worms.
Larus argentatus	3	2	June, July, August 1949	This paper	
Charadrius wilsonia wilsonia	4	2	July, 1949	This paper	
Ammospiza maritima macgillivrai	100	18	Summers 1949, 50 and 51	This paper	
Fish					
Opsanus tau	145	5	August 1901–1902	Linton 1905	Called *Distomum aduncum.* Type specimen #37161 U.S.N.Mus.
Balistes carolinensis	4	4	Summer 1939	Hopkins 1940	No eggs. "All mature."
Opsanus tau	25	9	Summer 1949	This paper	Very few eggs.
Sphaeroides maculata	6	2	Summer 1949	This paper	Immature.

THE ADULT

Gynaecotyla adunca (Linton, 1905) char. emend. (Fig. 1). Specific diagnosis: (Measurements in millimeters, based primarily on stained specimens.) With the characters of the genus. Oval to pyriform in shape, concave ventrally. Cuticle spinous to anterior level of testes; spines smaller towards posterior limits of distribution. Length of body ranges from 0.35 to 0.84 (av. 0.61). Width in region of testes from 0.22 to 0.39 (av. 0.26). Subterminal oral sucker, 0.04 to 0.06 (av. 0.05) in diameter. Short prepharynx 0.01 to 0.04 (av. 0.023). Pharynx 0.015 to 0.03 (av. 0.026). Relatively long esophagus 0.08 to 0.20 (av. 0.17) bifurcates just beyond anterior third of body. Intestinal crura relatively thick, usually straight, sometimes lobulated, extend to mid-level of testes. Arched cirrus pouch lies in transverse plane immediately behind intestinal bifurcation, anterior to the two acetabula. Seminal vesicle and part of ejaculatory duct contained within cirrus pouch; distal end of ejaculatory duct lies in genital atrium before it enters small, forked cirrus. Acetabula slightly post-equatorial. Right acetabulum 0.03 to 0.045 (av. 0.039). Left acetabulum usually larger, though not without exception; 0.04 to 0.06 (av. 0.047). Genital atrium 0.06 to 0.10 (av. 0.079), contains complex muscular *genital guide* with three distinct parts, two forked lobes and a small, forked cirrus. A fan-shaped extrinsic muscle mass runs from posterior margin of the cirrus pouch to base of genital guide. Cuticular plates evident on guide and cirrus. Genital

pore non-muscular, on ventral surface, antero-lateral to dextral sucker. Testes 0.05 to 0.110 (av. 0.07) in diameter, at anterior level of posterior body third. Vasa efferentia join to form vas deferens, dorsal to left acetabulum near end of cirrus pouch. Ovary more or less spherical, lateral to left acetabulum, 0.05 to 0.08 (av. 0.063). Seminal receptacle and Laurer's canal absent. Short oviduct expands into fertilization chamber before leading into oötype. Oötype region surrounded by Mehlis' gland, either completely dorsal to left acetabulum or on mid-line of body slightly posterior, but overlapping both suckers. Uterus entirely posterior to cirrus pouch. Metraterm enters genital atrium on median dorsal surface; distal end reinforced by heavy cuticular horseshoe-shaped plate. Vitellaria consisting of seven to nine large follicles on each side of body, posterior and slightly lateral to testes. Vitelline ducts join and form small vitelline reservoir before emptying into oötype. Embryonated eggs 0.017 to 0.021 (av. 0.020). Excretory pattern typical of genus, 2[(2+2)+(2+2)]. Excretory bladder variable in shape; pore located at posterior tip of body.

Hosts: *Crocethia alba* (Pallas), *Larus argentatus smithsonianus* Coues, *L. atricilla* Linn., *Charadrius wilsonia wilsonia* Ord, *Ammospiza maritima macgillivraii* (Audubon), *Opsanus tau* (Linn.), *Sphaeroides maculata* (Block and Schneider), *Balistes carolinensis* Gmelin.

Location in host: Small intestine.

Locality: Beaufort, N. C.

Type: From *Opsanus tau.* Deposited in U. S. Nat. Mus., Helminthological Collection. No. 37161.

DISCUSSION

In his original description, Linton (1905) interpreted the left acetabulum as a genital aperture, thereby completely reversing the true direction of the cirrus pouch and placing the base of the cirrus at the distal end. His drawings show the generic and specific characters more clearly than his description, although his failure to correctly interpret the presence of two acetabula, etc., influenced the described relative position of the cirrus pouch and other structures. Hopkins (1940) listed these discrepancies as "exceptions to Linton's description."

Data on worms from different hosts show a wide range of variations in size in different physiological environments. This variation in size between specimens grown in experimental hosts showed an interesting correlation with the number of worms recovered. For example, the worms were recovered in greatest numbers from the common tern; these fell well within the size ranges of worms taken from the herring gull which is undoubtedly a normal definitive host. The worms from the least terns, however, were larger when recovered and were not considered to be in good condition. When grown *in vitro,* the worms in poor cultures were also larger; this size difference may have been due to the fact that the worms were in poor condition and becoming moribund. They appeared as many tissue cells do after having been in a hypotonic solution. The worms from fish were consistently larger than those from birds. Different physiological environments would be expected to yield variations in size. In collections from so-called normal or natural hosts, the larger worms were those which were less active and were judged to be older worms losing their viability.

The arcuate structure, usually called the cirrus pouch, never contains the cirrus. The name is retained, however, in order to simplify terminology. It contains the seminal vesicle, a wide tube arising immediately after the vas deferens enters the pouch. The seminal vesicle extends approximately two-thirds the length of the pouch before it narrows sharply into the ejaculatory duct. The width of the seminal vesicle varies with the sexual activity of the worm. The prostate cells sur-

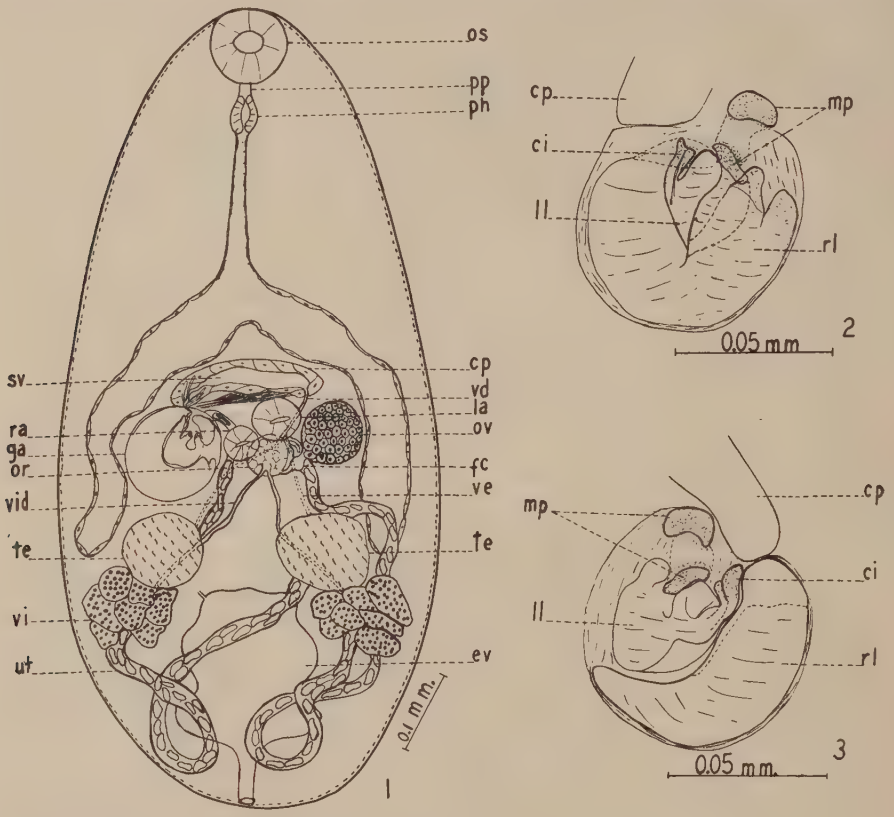

FIG. 1. Adult *Gynaecotyla adunca,* ventral view. Drawn from microprojection.
FIG. 2. Genital atrium, ventral view. Camera lucida drawing.
FIG. 3. Genital atrium, dorsal view. Camera lucida drawing.
Lettering: *ci,* cirrus; *cp,* cirrus pouch; *ev,* excretory vesicle; *fc,* fertilization chamber; *ga,* genital atrium; *la,* left acetabulum; *ll,* left lobe, genital guide; *mp,* metraterm plate; *or,* oötype region; *os,* oral sucker; *ov,* ovary; *ph,* pharynx; *pp,* prepharynx; *ra,* right acetabulum; *rl,* right lobe, genital guide; *sv,* seminal vesicle; *te,* testis; *ut,* uterus; *ve,* vas efferens; *vd,* vas deferens; *vi,* vitellaria; *vid,* vitelline duct.

rounding the seminal vesicle empty into the ejaculatory duct. The ejaculatory duct leads into the genital atrium and enters the cirrus, an outgrowth of a very muscular organ best described as a *genital guide.*

The genital guide consists primarily of two large muscular lobes extending from a broad basal part adjacent to the end of the cirrus pouch. The wall of the genital atrium separates the cirrus pouch from the modified copulatory organ. The

genital guide is often very active in living animals. The right lobe is shallowly forked at the free-moving and unattached distal end (Fig. 2). The tips of the two short projections bear rounded, spinous cuticular knobs; the outer margin of the right lobe is also spinous. This part of the genital guide is important in moving the eggs through the atrium and in directing the cirrus towards the uterine aperture. The left lobe of the guide is much more deeply forked (Figs. 2 and 3). These forked ends have cuticular tips and are much more slender than are those of the right lobe; the tips have been observed with a fully embryonated egg between them, apparently guiding it towards the genital pore. The structure which is the true cirrus is an outgrowth from the left lobe. The cirrus is a tubular structure, broad at the base, with two slender tips strengthened by cuticular plates (Figs. 2 and 3). Sperm have been observed in the duct within the cirrus, as well as in the seminal vesicle and ejaculatory duct, in worms shortly after excystment *in vitro*. The horseshoe-shaped cuticular plate surrounding the end of the metraterm is discontinuous ventrally; the two ventral ends are very prominent and aid in diagnosing this species (Figs. 2 and 3). The above described cuticular structures are constant in this species, but those other than the ventral ends of the metraterm plate are difficult to determine because of the varying positions of the two large lobes and cirrus. Linton mentioned "four stout hooks" at the base of the "muscular copulatory organ", but Rankin neither mentioned nor pictured any comparable structures. Yamaguti (1934 and 1939) also failed to note any constant cuticular structures in his generic and specific descriptions.

The so-called genital guide has been interpreted as the male copulatory organ, cirrus, or male papilla by Linton, Rankin, and Cable and Kuns. Detailed study by the writer leads to disagreement with those authors. Cable and Kuns (1951) call the comparable structure in the genus *Carneophallus,* a "male papilla." In their new species, *C. trilobatus,* the largest of the three lobes is penetrated by the ejaculatory duct, whereas in *G. adunca,* the smallest lobe contains the male duct, and the two larger lobes of the entire organ are functional in directing the cirrus and eggs. Other authors do not ascribe any function to the lobes besides that of containing the duct. This writer believes that such a modification of the male organ must involve other functions than those of copulation. In *G. adunca* no evidence has ever been found for cross fertilization. In fact, all evidence from the study of developing worms *in vitro* and from experimentally obtained adults leads to the conclusion that self-fertilization is the rule for this species.

In living worms ova were observed moving down the oviduct and at the same time sperm were seen in the expanded region of this duct. Vitelline material was added as eggs and sperm were being moved into the oötype. Shell material was laid down in the oötype and the completed egg was then forced out into the uterus. Egg formation was underway and completely shelled eggs were in the uterus ten to twelve hours after excystment. Eggs are yellow in color and are fully embryonated by the time they reach the metraterm. Embryonated eggs were being expelled from the genital atrium on the third day (77–80 hours) after excystment (Hunter and Chait, 1952).

The metacercariae are so similar to the excysted adults in size and structure that it is deemed unnecessary to give a detailed description of these larvae. The principal difference to be noted is the lack of development of the uterus. Upon

dissection from the cysts, and even before dissection, the genital guide is exceedingly active. The cuticular plates are very noticeable and diagnostic for the species. Measurements fall entirely within the size ranges given for the adults. The green glands of the intermediate host, *Uca pugilator,* are the organs most heavily infested, but cysts are also found throughout the hemocoel, in the gonads, and in the digestive gland of this crab. The cysts are not intimately involved with the host tissue as they readily fall out of the crab when its tissues are torn apart. As many as 150 cysts have been taken from one green gland. From the one selected collecting area (this study), seldom has an uninfected crab been found. Oval cysts, measured without coverglass pressure, ranged from 0.35 to 0.45 by 0.24 to 0.29, averaging 0.41 by 0.26 mm.

SUMMARY

Adult *Gynaecotyla adunca* (Linton, 1905) were obtained from experimental bird hosts which were fed encysted metacercariae taken from *Uca pugilator*. Data on the feeding experiments are given, as well as a redescription of the adult worm first described as *Distomum aduncum* by Linton (1905). Particular emphasis is placed on the complex structure (genital guide) within the genital atrium. Cuticular plates present on the muscular genital guide, cirrus, and metraterm are considered to be diagnostic for the species.

A list of reported hosts is given and the wide variety of hosts for this species is considered.

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THE RELATION OF *CULICOIDES* (DIPTERA: HELEIDAE) TO THE TRANSMISSION OF *ONCHOCERCA VOLVULUS**

COLVIN L. GIBSON[1] AND WERNER F. ASCOLI[2]

Apparently the first definite association of flies of the family HELEIDAE with the transmission of filarial infections was that made by Sharp (1927, 1928), who reported the development of *Acanthocheilonema perstans* in *Culicoides grahami* and *C. austeni* in the Cameroon, West Africa. The recent reports of Henrard and Peel (1949) and Chardome and Peel (1949) strongly suggest that Sharp was actually working with *Dipetalonema streptocerca* instead of *A. perstans,* as he believed, but his studies nevertheless were the first to establish definitely heleid flies as possible intermediate hosts of filarial worms. Sergent *et al* (1933) reported the discovery of unidentified microfilariae in *Holoconops mediterraneous* in Algiers. Buckley (1933, 1934) demonstrated that *Culicoides furens* is the intermediate host of *Mansonella ozzardi* on St. Vincent Island, British West Indies. George (cited by Causey, 1938) showed that certain species of *Culicoides* were capable of transmitting filarids of monkeys in Panama. Causey (1938) reported a specimen of *Culicoides peregrinus,* collected in Nakon Sri Tamarat, Siam, which was heavily infected with filarid larvae.

Steward (1933) first demonstrated the role of heleid flies in the transmission of filarial parasites of the genus *Onchocerca.* Working in England, he found that *Onchocerca cervicalis* of horses is transmitted by *Culicoides nebeculosus,* and possibly also by *C. obsoletus* and *C. parroti.* Dampf (1936a, 1936b, 1936c) found developmental forms of filarid larvae in three of 107 wild specimens of a hitherto undescribed heleid which Hoffman (1939) subsequently named *Culicoides filariferus.* The larvae were of two sizes which, according to Dampf, corresponded to *Onchocerca volvulus* (= *caecutiens*) and *O. cervicalis,* although only the former was known to occur in the region of Mexico where the infected flies were collected. Further elucidation of the role of *Culicoides* as an intermediate host of *Onchocerca* came from the work of Buckley (1938), in which it was shown by a very large series of dissections of both wild and experimentally infected flies that *Onchocerca gibsoni* of cattle in Malaya is transmitted by *Culicoides pungens.* Several other species of *Culicoides* may also be capable of transmitting these parasites, but Buckley's data are not conclusive. Vargas (1941) suggested that *Culicoides* might be involved in the transmission of *Onchocerca volvulus* because it has mouthparts adapted to the removal of microfilariae from the superficial layers of the skin, but he has reported no experimental work along this line.

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[2] Pan American Sanitary Bureau.

The foregoing studies, which definitely link various species of *Culicoides* with the transmission of at least four species of filarial parasites, were reviewed by Vargas (1945). In view of these results, and despite Sharp's report (1928) that *Onchocerca volvulus* did not develop in *Culicoides austeni* in Africa, it seemed advisable to determine whether gnats of the genus *Culicoides* might serve as intermediate hosts of American human onchocerciasis, in addition to the several species of *Simulium* already known to transmit the disease.

MATERIALS AND METHODS

The present investigation was carried on at Finca (Plantation) San Rafaél, near San Pedro Yepocapa, Department of Chimaltenango, Guatemala. This plantation is located in the endemic zone of onchocerciasis, and during the present investigation 14 per cent of the inhabitants of the plantation revealed microfilariae of *Onchocerca volvulus* in skin biopsies.

The work was carried on during a one-year period from December 1950 through December 1951, and thus embraced both the dry season and the rainy season. In order to determine the natural rate of infection, wild *Culicoides* were captured in test tubes as they alighted on human volunteers who were free of infection with *Onchocerca volvulus*. These volunteers were examined several times during the course of the investigation by means of skin biopsies, to be sure they were not carrying microfilariae which might infect the flies.

A subject who was heavily infected with *O. volvulus* and who repeatedly showed microfilariae in skin biopsies was used for experimental infection studies. He had been used constantly for two years in similar experiments with *Simulium*, and was known to be capable of producing infections in flies of that genus. Wild *Culicoides* were collected as they fed on the subject, by placing the mouth of a test tube over them and waiting until they had engorged, when they would fly to the bottom of the tube and could easily be confined. Only one fly was collected in each tube, as it became evident early in the study that crowding reduced the survival time. The inclusion in the tube of a strip of paper towelling for support materially increased the longevity of the flies.

The tubes were transported to the laboratory in canvas bags which usually were kept moist to reduce the temperature by evaporation. In the laboratory, they were kept in the dark in order to reduce activity, thereby prolonging their survival. The temperature in the laboratory was approximately 20° C. throughout the course of the experiments. The tubes were capped with rubber stoppers of the "Vacutainer" type which have a depression on the inner face into which a small pledget of absorbent cotton, previously moistened in sugar-water, could be inserted. The cotton was changed daily to provide food, maintain high humidity within the tubes, and prevent formation of molds. No other food was offered as preliminary trials indicated that subsequent blood meals, which the flies would take avidly, greatly reduced the survival time.

Flies collected from the uninfected volunteers were dissected as soon as possible after arrival at the laboratory. Flies which fed on the infected subject were examined twice daily and dissected as soon as they died. The head, thorax, and abdomen of each fly were placed in separate drops of physiological saline and dissected individually.

RESULTS

Four anthropophilic species of *Culicoides* were encountered during the present study: *C. paraensis* (Goeldi), *C. guttatus* (Coq.) and two hitherto undescribed species, *C. gibsoni* and *C. stigmalis*. Identifications were made by Dr. Willis W. Wirth of the Division of Insect Indentification, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, to whom we are deeply indebted. The two new species are described by Dr. Wirth in an adjoining publication. During the early part of the study the distinction between *C. paraensis* and *C. gibsoni* was not appreciated, and for a time the two together were designated as Species "B." It was later discovered that this complex consisted largely of *C. paraensis*, with about four per cent belonging to the new species *C. gibsoni*.

Culicoides paraensis was always the dominant and most annoying species present during our studies. It bites at any hour and under all weather conditions; some specimens will even bite during a heavy rain. About eight minutes are required for engorgement. Insect repellent ("Skat," dimethyl phthalate) prevents the flies from biting the areas covered by the repellent, but they will bite on any non-treated area (under a watchband, around the eyelids, in the ears, etc.). The minute size and persistence of this species make it particularly annoying.

Culicoides gibsoni is indistinguishable from *C. paraensis* in the field. It apparently bites under the same conditions as *C. paraensis*, but is found in very limited numbers.

Culicoides stigmalis is larger than *C. paraensis*, and is easily recognized in the field by its generally dark appearance. It bites most frequently between 4:30 and 6:00 P.M. with the sky partially overcast. The warmer the day the better, but the species never is present in large numbers. Engorgement time is about eight minutes.

Culicoides guttatus is about the same size as *C. stigmalis*, from which it can easily be distinguished in the field by its spotted wings. This species bites only during or immediately after a light rain. It has not been taken on sunny or overcast days. Its feeding time is much longer than that of the other species, averaging about ten minutes, and it does not engorge as fully as do the others. It is the only species of the four which will not resume feeding once it has been disturbed. *C. guttatus* is of particular interest in connection with the transmission of onchocerciasis, since Macfie (1948) believes that it is identical with *C. filariferus*, in which Dampf (1936a, 1936b, 1936c) found developmental forms of filarid larvae, presumably *Onchocerca volvulus* and *O. cervicalis*, in Chiapas, Mexico.

Table 1 shows the results of dissections of *Culicoides* collected from uninfected subjects (natural infection) and from a subject heavily infected with *Onchocerca volvulus* (experimental infection). Although no evidence of a natural infection was found in any of the four species, the numbers of *C. guttatus* and *C. gibsoni* dissected are too small to be significant.

In attempts to cause experimental infection, *C. paraensis* proved completely incapable of ingesting microfilariae. The same was true of *C. gibsoni*, but the number of flies (10) was too small to justify a definite conclusion. This species appears to be extremely rare; it is therefore unlikely that it is involved in the transmission of human onchocerciasis in the area under study.

In contrast, *Culicoides stigmalis* readily ingested microfilariae from the infected subject. Subsequent to exposure, 19.0 per cent or 58 of 305 flies of this species,

were found to harbor from one to 16 filarid larvae. In view of the failure to find natural infections in 220 wild flies of this species, there can be no doubt that these larvae came from the subject used in the experimental feedings. The species proved unable to support development of the filarid larvae, however. All except one of the infected flies died within two days, and none of the microfilariae in these flies had undergone perceptible development. The one surviving fly, which lived until the seventh day after the infective blood meal, contained a single microfilaria in the abdomen. This larva measured 320 μ by 8 μ and thus had undergone slight growth. (Microfilariae in human skin vary from 210 μ to 300 μ in length by 7 μ to 8 μ in width.) Although this larva had grown somewhat it showed very little differentiation, whereas six-day larvae in *Simulium* have the various organs well developed (Gibson, 1951). Moreover, the larva seemed to be degenerating, as evidenced by extremely feeble movements and the extensive vacuolation of the cytoplasm of the cells. It therefore appears evident that *C. stigmalis* is not a suitable intermediate host for *O. volvulus* even though microfilariae are readily ingested.

TABLE 1—*Results of dissections of* Culicoides *collected on uninfected subjects (natural infection) or fed on a heavily infected subject (experimental infection).*

Species of *Culicoides*	NATURAL INFECTION			EXPERIMENTAL INFECTION		
	Number of Flies Dissected	Flies Containing Microfilariae		Number of Flies Dissected	Flies Containing Microfilariae	
		Number	Percentage		Number	Percentage
C. gibsoni	10	0	0.0	10	0	0.0
C. guttatus	26	0	0.0	47	1	2.1
C. paraensis	491	0	0.0	385	0	0.0
C. stigmalis	220	0	0.0	305	58	19.0
Species "B"*	182	0	0.0	182	0	0.0
TOTAL	929			929		

* Species "B" represents a complex composed of approximately 96 per cent *C. paraensis* and four per cent *C. gibsoni* (see text).

One of 47 specimens of *Culicoides guttatus* (2.1 per cent) contained a single living microfilaria in the abdomen when it died 15 hours after feeding on the infected subject. On the basis of these meager data, no conclusion can be drawn regarding the potentiality of this species as a transmitter of *O. volvulus*. In view of Macfie's belief that this is the species in which Dampf found naturally-occurring infections (see above), it would be advantageous to examine a larger series of specimens, although the rarity of the species as noted during the present study would indicate that it is of little, if any, importance as an intermediate host of human onchocerciasis in the Yepocapa region of Guatemala.

Fig. 1 graphically illustrates the effect of ingested microfilariae on the survival of *Culicoides stigmalis* in the laboratory. The rapid death of infected flies, as compared with those which fed on the same subject but did not ingest microfilariae, substantiates the previously indicated conclusion that this species is not a suitable intermediate host for *O. volvulus*. There was no correlation between survival time and the number of microfilariae ingested.

SUMMARY

1. Four anthropophilic species of *Culicoides* have been found in the San Pedro Yepocapa region of the onchocerciasis zone in Guatemala. These are *C. paraensis, C. guttatus, C. gibsoni,* and *C. stigmalis.*

2. No evidence of natural infection with filarid larvae was found in dissections of 929 wild flies of the four species. The series of dissections of *C. guttatus* and *C. gibsoni* are too small to permit valid conclusions, however.

3. Experimental infection studies were made by capturing 929 flies, representing all four species, as they fed on a human volunteer heavily infected with microfilariae of *Onchocerca volvulus*. *C. paraensis* was incapable of ingesting microfilariae. In the case of *C. stigmalis,* 19.0 per cent became infected but the ingested microfilariae underwent no perceptible development. One of 47 specimens of *C. guttatus*

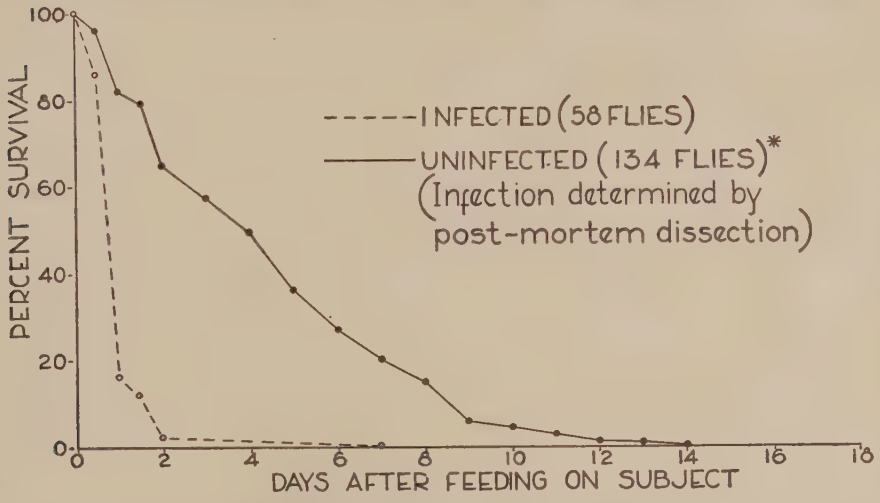

FIG. 1. Survival of *Culicoides stigmalis* in the laboratory after feeding on a subject heavily infected with microfilariae of *Onchocerca volvulus,* showing the increased mortality of flies which ingested microfilariae.

* Survival data were not available for some of the *uninfected* flies recorded in Table 1. These are not included in the curve.

which fed on the infected patient ingested microfilariae, but the fly died within 15 hours. No flies of *C. gibsoni* ingested microfilariae, but the number of specimens (10) available is too small to allow definite conclusions.

4. Flies of the species *C. stigmalis* which ingested microfilariae from the infected patient died much more rapidly than did uninfected flies. This fact, together with the failure of microfilariae to undergo development, indicates that this species is not a suitable intermediate host for *O. volvulus*. It is further concluded that *C. paraensis* is unsatisfactory as an intermediate host because of its inability to ingest microfilariae. *C. guttatus* and *C. gibsoni* may be potentially capable of transmitting human onchocerciasis, but their rarity makes it most unlikely that they are actually involved.

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POMATIOPSIS LAPIDARIA, ITS OCCURRENCE IN THE WASHINGTON, D. C. AREA AND ITS LABORATORY REARING IN COMPARISON TO THAT OF *ONCOMELANIA* SPP.[1]

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Many native snails of the United States were tested during and following World War II to determine their capacity to serve as intermediate hosts for *Schistosoma japonicum,* the Oriental blood fluke. *Pomatiopsis lapidaria* Say, an amnicolid found widely distributed in the eastern half of the United States, was the only species incriminated. Stunkard (1946) found that partial development of *S. japonicum* might occur in this species and Ward, Travis, and Rue (1947) made similar observations. Complete development of *S. japonicum* in *P. lapidaria,* with the production of cercariae infective for hamsters, was reported by Berry and Rue (1948). Since then, in the Laboratory of Tropical Diseases of the National Institutes of Health, more than 2,000 specimens of *P. lapidaria* have been exposed to miracidia of *S. japonicum.* Only 5 have been observed to shed cercariae. From this, it would seem that *P. lapidaria* is poorly adapted to serve as intermediate host for *S. japonicum.* However, in the course of this work considerable information has been obtained on the habits and habitats of *P. lapidaria,* and because it has some potentiality as a vector of *S. japonicum* this information is of interest.

Pomatiopsis lapidaria is broadly adapted as an intermediate host for trematodes. It is known to serve as intermediate host for *Paragonimus kellicotti,* the North American lung fluke (Ameel 1932) ; *Nudacotyle novicia,* found in the bile duct of the meadow mouse (Ameel 1944) ; and *Euhryhelmis monorchis,* normally infecting the mink (Ameel 1938). In addition *P. lapidaria* is known to harbor two cercariae, *Cercariae geddesi* (Ameel 1939) and *Cercariae pomatiopsidis* (Stimson 1865 and Ameel 1939), the adults of which are unknown.

The range of *P. lapidaria* is from "Minnesota east through Ontario to southern New York. South to Alabama and Texas. Eastern Atlantic Seaboard from Pennsylvania south to Virginia. No records in New England, the Carolinas, Georgia, or Florida" (Abbott 1948),

There are conflicting concepts of the habits of *P. lapidaria.* Stimson (1865), Pilsbry (1896), and Shimek (1930a, 1930b) considered *P. lapidaria* to be a land snail. Walker (1918) pointed out that this species is "terrestrial or rather semi-amphibious in habit, being always found near but not in the water." Baker (1930, 1931) regarded *P. lapidaria* as essentially amphibious, pointing out that even though it is most frequently found in damp areas along the edge of streams, it does not crawl to higher ground when the area becomes flooded. He also reported finding this species on the bottom of streams of normal depth. Ameel (1938) reasoned that

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since *P. lapidaria* harbors at least 5 different kinds of cercariae which require a snail host with a definite aquatic phase, it must be amphibious. In the Washington, D. C. area its amphibious nature is apparent.

The laboratory rearing of snails is necessary for experimental studies on the intermediate host phase of trematode life cycles, especially if the research is undertaken outside endemic areas. The rearing of such snails has been accomplished with varying degrees of success. Many species of aquatic snails have been successfully maintained in the laboratory for indefinite periods, but the maintenance of permanent colonies of the amphibious snail hosts of *S. japonicum* is a difficult undertaking. Vogel (1948), however, successfully bred *Oncomelania hupensis,* a snail host of *S. japonicum* in China, for more than 10 years in his laboratory in Germany. Ward, Travis, and Rue (1947) described the early work at the Laboratory of Tropical Diseases in establishing and maintaining *O. quadrasi, O. hupensis,* and *O. nosophora.* Satisfactory results were obtained only with *O. quadrasi.* Later, Berry and Rue (1947) were successful in rearing *Pomatiopsis lapidaria* in the laboratory. Subsequent experimentation led to more satisfactory results, and at present colonies of 5 species of amphibious snails are being maintained: *Pomatiopsis lapidaria,* from the Washington, D. C. area, maintained since 1947; *O. quadrasi,* from the Philippines, since 1945; *O. hupensis,* from China, since 1948; *O. nosophora,* from Japan, since 1949; and *O. formosana,* from Formosa, since September 1950. Additional specimens of *O. nosophora* were received from Japan in November 1950 and in October 1951. The colonies of the other 3 species of *Oncomelania* have been maintained without addition of wild specimens.

Habitats of *Pomatiopsis lapidaria* in the Washington, D. C. area

According to collection records of the Division of Mollusks, United States National Museum, *Pomatiopsis lapidaria* has been collected in a number of localities in the Washington, D. C. area. The present writer has been able to find this species, however, in only 3 areas, all of which are located on the banks of the Potomac River. It occurs in large numbers on the west side of the river about one-quarter of a mile downstream from Key Bridge. It occurs also, but in smaller numbers, on the east side of the river near Fletcher's Boat House about two and one-half miles upstream from Key Bridge, and at Fox's Ferry approximately 7 miles downstream from Key Bridge.

The Key Bridge area, situated at the foot of a steep bank, extends for approximately 300 yards along the edge of the river and is about 60 feet wide. The soil is composed of mud and sand near the river and changes to a sandy loam toward the upper edge. The pH of the soil ranges from 7.0 to 7.6. Vegetation, composed of many types of marsh plants and grasses, covers the entire area; a border of large trees along the upper edge of the flat area provides shade from the afternoon sun. Driftwood, continually washed in from the river by tide action, is strewn over most of the area. The 2-foot change in water level at this point, due to tide action, results in the area being submerged for several hours twice each day, while at low tide the area is drained.

The Potomac River water was found to be alkaline in a series of pH determinations made during the summer of 1951; samples taken near Key Bridge at high

tide were 7.8, 8.1, 8.4, 8.2, 8.1, and 8.3 with an average of 8.2, and samples taken at low tide were 8.5, 8.6, and 8.3, average 8.4.

Chemical analysis of a water sample taken at low tide on June 8, 1951, revealed the following:[3]

	Parts per million		Parts per million
Total dissolved solids (103° C.)	156.0	Sodium and potassium (calculated as Na)	5.4
Loss on ignition	21.4	Carbonate (CO_3)	0.0
Fixed residue	134.6	Bicarbonate (HCO_3)	87.8
Silica (SiO_2)	7.2	Sulfate (SO_4)	30.9
Iron (Fe)	0.1	Nitrate (NO_3)	2.2
Aluminum (Al)	0.1	Chloride (Cl)	4.0
Calcium (Ca)	28.6	Phosphate (PO_4)	0.0
Magnesium (Mg)	7.9		

The low chloride level of this sample indicates that the river water is not brackish. The Coast and Geodetic Survey records show that the Potomac River rarely becomes brackish as far up as Indian Head, Maryland, which is more than 25 miles downstream from Key Bridge, and the river has never been known to be brackish as far up as Washington, D. C.

The other two localities, both of which are located on the east bank of the river, one upstream and the other downstream from Key Bridge, are similar in most respects to the area near Key Bridge. They are flat vegetated areas along the edge of the river which are submerged at high tide and are drained at low tide. The area at Fox's Ferry is at least three-fourths mile long and up to one-fourth mile wide, and the Fletcher's Boat House area is just slightly smaller. The pH of the soil and river water at these points was found to be approximately the same as that at the area near Key Bridge.

It is of interest to note that the Fox's Ferry area is located only one-half mile downstream from the District of Columbia sewage disposal plant. Thus, if infective stages of *S. japonicum* should be discharged from the sewage disposal plant in sufficient numbers, there would be a chance for the *P. lapidaria* in this area to become infected.

Habits of *Pomatiopsis lapidaria* in the Washington, D. C. area

Field observations on *P. lapidaria* in the Washington, D. C. area during the past 3 years revealed the habits to vary according to the seasons of the year. With the arrival of cold weather in the fall the snails seek protected places to spend the winter. Some crawl into depressions in the ground and others into cracks in decaying driftwood. Here they retract into their shells and close the openings with their opercula. The winter is then passed in a state of hibernation. The first warm days of spring awaken the snails into activity and almost immediately many male and female snails begin to copulate. Eggs are laid and after several weeks young snails appear. These young snails grow rather rapidly and by mid-summer it is unusual to find any juvenile *P. lapidaria.* Thus a big wave of young snails appears in the spring, but reproduction does not continue throughout the summer.

[3] Determinations made by Dr. Elias Elvove of the National Institute of Dental Research.

During the first part of September many snails are again found in copulation, and by the end of the month a second wave of young snails appears. Young snails are not as numerous at this time, however, as they are in the spring, and they do not have sufficient time to become mature before cold weather forces them into hibernation. This results in the finding of 2 sizes of snails when hibernation is terminated in the spring.

During the summer *P. lapidaria* feeds almost continually by browsing over decayed driftwood and vegetation. Its diet, apparently, is composed entirely of decaying organic material. Food material is ingested by the rasping action of the radula and fecal material is deposited in the form of small egg-shaped masses.

Twice each day tide action on the river results in the flooding of the areas along the Potomac where *P. lapidaria* is found. When the areas are flooded it is quite obvious that the snails feel at home in the water, for they make no attempt to crawl to higher ground or up the sides of plants to escape the water. This supports previous statements concerning the amphibious nature of *P. lapidaria.*

In attempting to establish colonies of 4 species of snails belonging to the genus *Oncomelania,* as well as *Pomatiopsis lapidaria,* efforts were made to duplicate conditions found in the natural habitat of each species. The procedures now used are described briefly below.

Containers used for the breeding of Oriental snail vectors of *Schistosoma japonicum* as well as *Pomatiopsis lapidaria* were designed to satisfy the amphibious nature of the snails. They are rectangular in shape and are arranged with a land portion at one end, or at one side, and a water portion at the other. Vogel (1948) called this type of an arrangement an aquaterrarium because it simulates the junction of a body of water and the land along its edge. The details of the arrangement of these containers are varied to simulate the natural habitat of the particular species of snail. For example, the aquaterrarium for *Oncomelania hupensis* is built to simulate the sides of irrigation canals whose banks are sharply inclined, while that for *O. quadrasi* is built to reproduce the flat-banked slow-running streams as found in marsh-land. The soil used in preparing aquaterraria is a sandy loam found in an area on the edge of the Potomac River where *Pomatiopsis lapidaria* occurs. Decaying vegetation from the same area is strewn over both the land and water portions of the containers and tap-water, after it is allowed to stand in large open glass containers for several days, is added to the desired level. Continuous aeration of the water is provided to increase the oxygen content and thus retard fouling of the water. When these arrangements are completed, the aquaterraria are allowed to stand for several days before the snails are introduced.

In order to maintain a high moisture content in the aquaterrarium, the top is covered with a glass plate except for a small crack for ventilation. A device (DeWitt 1951), which is essentially an atomizer that is operated by compressed air and produces an exceptionally fine spray, is attached to the water-end of the aquaterrarium by means of a rubber suction cup. Each morning the air is turned on and a fog-like mist fills the container. After a few minutes moisture collects on the glass sides as well as on the grass and decaying vegetation. The air is then turned off and the resulting condition in the aquaterrarium resembles a heavy dew. The use of this device has proved to be beneficial in the breeding of these amphibious snails.

Food for the snails is provided mainly by the decaying vegetation in the aquaterrarium. However, powdered commercial fish food (ingredients: crab meal, liver, shrimp, beef, fish, mosquito larvae, fish bone, wheat cereal, and mineral supplements) is sprinkled on the ground near the edge of the water once or twice a week. All 4 species of *Oncomelania* as well as *Pomatiopsis lapidaria* readily feed on this food.

The 4 species of *Oncomelania* maintained in this laboratory came from areas with different climatic conditions and this may account in part for differences in their natural reproductive periods. *O. quadrasi* in the Philippines lives in a tropical environment, and the seasonal variation in temperature is only slight. Reproduction continues throughout the year, but it is more marked during the rainy season. *O. nosophora* in Japan and *O. hupensis* in China, however, are subjected to freezing weather in the winter, and with these species reproduction is limited to the spring and early summer months. *O. formosana* in Formosa is not subjected to as severe winter weather, but it reproduces only during the spring and early summer months. In the laboratory, these 4 species of *Oncomelania* as well as *P. lapidaria* have been maintained at a constant temperature of 26° to 28° C. throughout the year. Under these conditions all 5 species reproduce without regard to the seasons.

The time required for newly hatched young snails to mature and reproduce under these conditions is a follows: *Oncomelania hupensis,* about 12 months; *O. nosophora,* 6 to 7 months; *O. formosana,* 6 to 7 months; *O. quadrasi,* about 3 months; and *Pomatiopsis lapidaria* not definitely known but probably about 12 months.

The close relationship of *P. lapidaria* to the species of the genus *Oncomelania* that serve as intermediate hosts for *Schistosoma japonicum* is indicated not only by their morphological similarity and their ability to serve as hosts for *S. japonicum* but also by similarities in their habits and in the laboratory conditions under which they will grow and reproduce. The techniques successfully employed to maintain laboratory colonies of the Oriental snail hosts of *S. japonicum* are not different from those used to maintain colonies of *P. lapidaria.* This suggests the possibility that if snails belonging to the genus *Oncomelania* were introduced into areas of the United States where *P. lapidaria* normally occurs, they might become established.

The possibility that the successful establishment of such snails in the United States would result in the transmission of schistosomiasis in this country is of course limited by numerous factors such as the number of infected final hosts and the sanitary practices in general use.

The development of techniques for maintaining permanent laboratory colonies of amphibious snails has made it possible to establish the life-cycle of *S. japonicum* in laboratories outside the Orient. This has opened up the way for broader studies on the basic biological relationships between this medically important parasite and its mammalian and snail hosts.

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of schistosomiasis on the other side of the Pacific. Without this cooperation these studies would not have been possible.

SUMMARY

Pomatiopsis lapidaria, a potential intermediate host of *Schistosoma japonicum,* is found in rather large numbers in 3 marshy areas along the edge of the Potomac River in the vicinity of Washington, D. C. The habits and habitats of this snail as found in these areas are described. Laboratory rearing techniques are presented whereby *P. lapidaria* and 4 species of *Oncomelania* may be maintained in aquaterraria.

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STUDIES ON THE TREMATODE FAMILY CYATHOCOTYLIDAE POCHE, 1926, WITH THE DESCRIPTION OF A NEW SPECIES OF *HOLOSTEPHANUS* FROM FISH AND THE LIFE HISTORY OF *PROHEMISTOMUM CHANDLERI* SP. NOV.[1]

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INTRODUCTION

Athough digenetic trematodes of the family CYATHOCOTYLIDAE have received considerable attention in Europe and Asia, this group has been neglected until very recently in North America. This may be due largely to the small size of the worms and the ease with which they could be overlooked. That cercariae of freshwater cyathocotylids occur in the United States was known before any adults were reported. Cable (1938) described *Cercaria kentuckiensis,* a typical cyathocotylid larva of the Vivax group of furcocercariae (Sewell, 1922). The only other freshwater larvae of this type reported from the United States are the cercaria of *Linstowiella szidati* (Anderson, 1944) Anderson and Cable, 1950, from Indiana, and *Cercaria yankipenensis* Goodman, 1951, from Reelfoot Lake, Tennessee.

To date only five adult cyathocotylids have been reported from North America. These are: *Neogogatea bubonis* Chandler and Rausch, 1947; *Neogogatea pandionis* Chandler and Rausch, 1948; an unnamed species reported by Cable and Vernberg (1949); *Mesostephanus longisaccus* Chandler, 1950; and *Linstowiella szidati* cited above.

The cyathocotylid species reported in a brief abstract by Cable and Vernberg (1949), was referred by them to the genus *Cyathocotyloides,* which is here regarded as a synonym of *Holostephanus* for reasons that will be given later. This species is not only the first of the genus to be reported from North America, but also the only representative of the STRIGEOIDEA known to utilize a fish as the natural definitive host; all other described members of this superfamily occur in birds, mammals, or less often, reptiles. At the time this unusual species was discovered in catfish from the Wabash River, two cercariae of the Vivax type had been collected from that stream. Since one of these was known to be the larva of *Linstowiella szidati,* it seemed possible that the other might be the cercaria of the species occuring in fish. This possibility seemed further strengthened by the fact that the species in the fish was the only adult cyathocotylid other than *L. szidati* known to occur in the locality. For this reason, and because of the general lack of information concerning the group in the United States, the present study was undertaken.

One of the major contributions of studies on the life histories of digenetic trematodes is the light such studies throw on relationships. There is special need for such information concerning the CYATHOCOTYLIDAE, the taxonomy of which

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has been based entirely on adult morphology with little or no consideration of the larval stages. Adult morphological characteristics show many variations and intergradations which render objective analysis extremely difficult. For that reason, the taxonomy of the CYATHOCOTYLIDAE is at present in a state of confusion with wide disagreement among workers with this group.

The species of Vivax-type cercaria under investigation was at first identified tentatively as *Cercaria kentuckiensis.* Since this larva does not emerge in numbers until June in the Lafayette region, infected snails were obtained from southern Indiana for use earlier in the year. Critical comparison of cercariae from the two regions has revealed that they are distinct species, the southern one being *C. kentuckiensis,* while the northern form is actually new and is described in this paper. Thus two cyathocotylid larvae with unknown life cycles were available to the writer for investigation.

Although fifty-four species of adult cyathocotylids have been reported, the life-histories of only nine of these have been described in detail. Those of *Cyathocotyle orientalis* elucidated by Yamaguti (1940), *Prosostephanus industrius* by Tang (1941), and *Szidatia joyeuxi* by Joyeux and Baer (1941) should be added to the life cycles listed by Anderson and Cable (1950).

OBSERVATIONS

Experimental Studies on Life Cycles

Cercaria kentuckiensis, one of the two species of Vivax type larvae available to the writer, was obtained from snails collected from Clifty Creek in southern Indiana and identified as *Goniobasis livescens* Menke by Dr. F. Hass, Chicago Natural History Museum. The other cercaria, an undescribed species, develops in *Pleurocera acuta* (Say) from the Wabash and Tippecanoe Rivers in the Lafayette vicinity.

Although the two cercariae are very similar, there are morphological differences which will be given below. Attempts to demonstrate their life cycles also revealed that the two utilize different intermediate hosts. *Cercaria kentuckiensis* was found to encyst and develop normally only in a single species of darter, although it would penetrate certain other fishes. Great difficulty was experienced in keeping infected darters alive in the laboratory and for that reason metacercariae presumed to be infective were available for but a single feeding experiment with a nestling green heron in an unsuccessful attempt to obtain the adult stage.

In determining the second intermediate host of the undescribed cercaria, all available species of minnows and smaller fishes were collected from the same habitat as that of infected snails and exposed to large numbers of cercariae. They were found to penetrate and encyst only in two species of bass, the large-mouth, *Huro salmoides,* and the small-mouth, *Micropterus dolomieu.* Examination of fingerlings of these species from the area revealed natural infections with metacercariae of this trematode.

To obtain infective metacercariae, snails shedding cercariae in large numbers were placed in the aquarium with young bass. Fish up to 25 mm. in length were killed by massive infections, but larger ones survived them. At the end of three weeks, the metacercariae were presumed to be infective because the primordia of adult structures were apparently undergoing no further development. Such

metacercariae were used in attempts to determine the definitive host and obtain adult worms by feeding experiments.

A small specimen of *Natrix sipedon* was fed an infected bass and examined after 36 hours; thirty-five "immature adults" were recovered from the small intestine. These worms were not considered to be fully mature since no eggs were present, although the reproductive systems were well-differentiated. One less well-developed specimen was recovered from the intestine of a miller's thumb, *Cottus bairdii,* killed 18 hours after being fed metacercariae. Three channel catfish, *Ictalurus punctatus,* were fed metacercariae and killed at intervals of 10, 24, and 48 hours respectively. Three excysted metacercariae were removed from the catfish examined 10 hours after the feeding, but the remaining fish were negative.

A rat, 10 baby chicks, a nestling green heron, *Butorides virescens,* six water snakes, a single specimen each of the turtles *Sternotherus odoratus, Pseudemys troosti,* and *Chelydra serpentina,* four frogs, *Rana clamitans,* and four yellow mudcats, *Ameiurus* sp., were subjected to feeding experiments, but without success.

The natural definitive host has not been found. However, it seems probable from the results above that the natural host is a poikilothermal animal. The trematodes persisted longest in the water snake, *Natrix sipedon,* and those recovered from that source were developed sufficiently to be identified as a new species of *Prohemistomum* for which the name *Prohemistomum chandleri* sp. nov. is proposed.

Description of Stages in the Life History of *Prohemistomum chandleri* sp. nov. (all measurements in millimeters).

The Sporocyst (Fig. 1)

Sporocysts producing cercariae occur in the digestive gland of *Pleurocera acuta.* They are elongate forms, and mature ones measure 3.360×0.277 to 4.820×0.292.

The anterior end is distinguished by its active exploratory movements and its shape, which is much narrower and more pointed than the rather broadly rounded posterior end. Also the birth pore near the anterior end forms a distinct cleft which is more prominent in smaller sporocysts than in larger ones. Mature cercariae were observed to emerge through this opening.

What is believed to have been the mother sporocyst was seen once. It was found in the branchial region rather than the liver. Although it was not a complete specimen and appeared empty, a few flame cells were still beating. The specimen was found early in May, and further attempts to find mother sporocysts were unsuccessful.

The daughter sporocysts are morphologically indistinguishable from those of *Cercaria kentuckiensis* and lack the prominent transverse muscle bands characteristic of sporocysts of such species as *Linstowiella szidati* and *Cercaria indica XV* Sewell.

The Cercaria (Fig. 2)

Specific diagnosis: (measurements from 10 living specimens under light cover glass pressure.) Longifurcous, pharyngeate distome cercaria with characteristics of Vivax group and subgroup. Body flattened dorso-ventrally, concave ventrally, oval or pyriform in shape; length 0.304–0.321 (average, 0.312), width 0.195–0.218 (average, 0.197); spinose except ventral surface of posterior region, spination especially conspicuous near the oral sucker. Cuticula 0.002 thick.

Tail inserted dorsally; length of tail stem 0.468–0.491 (average, 0.476), width 0.070–0.085 (average, 0.077). Furcal length 0.328–0.390 (average, 0.365); dorsal and ventral furcal fin-folds continuous around tips of furcae. Prominent caudal bodies arranged in two longitudinal rows, one on each side of the main excretory tubule in tail stem. Oral sucker pyriform, protrusible, with terminal opening; length 0.072–0.086 (average, 0.079), width 0.056–0.070 (average, 0.065); prepharynx lacking; length of pharynx, 0.020–0.025 (average, 0.022), width 0.023–0.027 (average, 0.025); length of esophagus 0.019–0.025 (average, 0.022). Intestinal ceca conspicuous, extending to the level of the excretory vesicle. Excretory vesicle and tubules typical of the Vivax Group. The collecting tubule on each side arises from the main lateral tubule slightly posterior to its junction with the transverse commissure and divides into anterior and posterior secondary tubules each of which receives the capillaries of three groups of three flame cells each. The posterior most flame cell group on each side is situated in the tail stem. The flame cell formula accordingly is $2[(3+3+3)+(3+3+3)]=36$ flame cells with six in the tail stem. Excretory pores at tips of the furcae.

Host: Pleurocera acuta (Say).

Locality: Tippecanoe and Wabash Rivers, Lafayette, Indiana.

Of 22,790 snails examined, 44 or 0.2%, were found to be infected with this cercaria. Shedding of cercariae was observed in the laboratory only during warm weather, from late May until early October.

The cercaria responds to shadowing by brief periods of swimming and is positively phototrophic, especially during the first few hours after emergence. The larvae are very active; they swim tail-first in a zig-zag path to the surface of the water and then slowly sink to the bottom with the body downward, the tail stem bent at an angle, and with furcae spread. They will attempt to penetrate moving objects and seem to be attracted to them. In the laboratory they rarely live longer than twenty-four hours after emergence.

The cercaria of *Prohemistomum chandleri* is very similar to *Cercaria kentuckiensis* (Fig. 3) with which it is easily confused. Both of these cercariae have been determined in the present study to have 15 pairs of flame cells instead of 14 reported by Cable (1938) for *C. kentuckiensis*. Comparison of the two has revealed several morphological differences. *Cercaria kentuckiensis* is significantly smaller than the larvae of *P. chandleri*. In *C. kentuckiensis* the anterior end of each lateral excretory canal gives rise to a lateral and a median branch at the esophageal level; the right and left median branches converge and may or may not unite. In the cercaria of *P. chandleri*, an inconspicious unbranched continuation of each lateral tubule extends anteriad beyond the junction with the cross commissure. The acetabular primordium of *C. kentuckiensis* is markedly smaller than that of the cercaria of *P. chandleri*. Conspicuous caudal bodies are present in this species while they are relatively inconspicuous in *C. kentuckiensis*.

The embryology of the excretory system was not traced in the cercariae studied, since this process is already well described for certain of their type. The accounts of Komiya (1939) and Anderson and Cable (1950) on development of the cercarial excretory pattern in *Paracoenogonimus ovatus* and *Linstowiella szidati* respectively are in exact agreement and may be taken as typical of the group, although they differ in certain respects from the observations of Looss (1896) on *Cercaria vivax* Sonsino.

The Metacercaria

The cysts of *Prohemistomum chandleri* were found distributed in the superficial muscles as well as adjacent to the vertebrae of infected fish. Cysts also were recovered from the gill region, from beneath the epidermis at the anterior tip of

the head, and from the tissues around the eyes but were never observed within the eyeball itself.

Forty-eight hours after encystment, the metacercaria has a single cyst wall; it was impossible to make out internal morphology at this time due to the extreme vacuolation. At 48 hours the cysts averaged 0.344 × 0.314 mm.

In older cysts, two distinct walls are visible, an innermost hyaline one of parasite origin, and an outer adventitious layer. The latter is thin, elastic, and yellowish in color. Between the two cyst layers, there is a space of irregular width filled with a granular substance. Sometime during the third week, melanophores appear in the area around the outer layer.

The metacercaria apparently becomes infective in about three weeks; at that time primordia of adult structures cease to develop further and the reserve excretory system is clearly defined. The lateral and median tubules are greatly enlarged and contain many refractive excretory granules. The cysts at this stage are round or oval, measurements averaging 0.606 × 0.475 mm., and have almost exactly the internal appearance that Anderson and Cable (1950) figured for the metacercaria of *Linstowiella szidati.*

The Adult (Fig. 4)

Specific diagnosis: with the characters of the genus *Prohemistomum.* Body pyriform, conspicuously spinose, ventral concavity shallow, its margin blending with the forebody anteriorly. Caudal papilla visible in living specimens, but not evident in whole mounts. Measurements from 10 specimens not quite sexually mature are as follows: Length 0.423–0.694 (average, 0.487); maximum width 0.292–0.365 (average, 0.315). Oral sucker wider than long, measuring 0.035 × 0.036 to 0.051 × 0.035 (average, 0.042 × 0.036). Length of pharynx 0.016–0.026 (average, 0.021). Esophagus moderately long, measuring 0.035–0.045 (average, 0.039). Ceca extend almost to posterior end of body. Ventral sucker slightly anterior to tribocytic organ, feebly developed, 0.024 × 0.026 to 0.032 × 0.035 (average, 0.027 × 0.028). Tribocytic organ in posterior one-third of body, poorly developed, 0.096 × 0.088 to 0.113 × 0.112 (average, 0.106 × 0.103), apparently not eversible. Testes in posterior part of body, tandem in arrangement, slightly to one side of body, and wider than long with anterior testis overlapping tribocytic organ. Anterior testis measures 0.072 × 0.047 to 0.073 × 0.031 (average, 0.073 × 0.043); posterior testis 0.075 × 0.044 to 0.120 × 0.044 (average, 0.097 × 0.044). Cirrus sac extends to level of anterior testis, measuring 0.105 × 0.018. Ovary 0.026 in diameter, overlapping anterior margin of tribocytic organ.

Host: (Experimental) *Natrix sipedon* Linnaeus.

Site: Small intestine.

Only a few species of *Prohemistomum* have been described. The genus was erected by Odhner (1913) for the reception of *P. spinulosum* whose larva later proved to be *Cercaria vivax* Sonsino, a name having priority over *P. spinulosum* Odhner. Mehra (1947) described *P. odhneri,* a single specimen of which he found in an Indian hawk. He also redefined the genus and reduced the genera *Linstowiella* and *Paracoenogonimus* to synonomy with *Prohemistomum.* Life cycles have been described for two species of *Linstowiella, L. viviparae* by Szidat (1933) and *L. szidati* by Anderson and Cable (1950). In both the cercariae are anacetabular and lack furcal fin-folds, whereas the larvae of *Prohemistomum vivax* and *Prohemistomum chandleri* are acetabular and have dorsal and ventral fin-folds continuous around the tips of the furcae. These larval dissimilarities, coupled with the fact that the adults of *Linstowiella* are anacetabular, afford as valid a basis for separating the genera *Prohemistomum* and *Linstowiella* as exists for the distinction of any other two genera in the family Cyathocotylidae. The genus *Paracoenogonimus,* has at present a single species, *P. ovatus,* the life cycle of which was

well described by Komiya (1939). The cercaria of *P. ovatus* is also anacetabular and lacks furcal fin-folds; the adult may be distinguished from *Prohemistomum* by the arrangement of the testes, which are obliquely situated on opposite sides of the body in *Paracoenogonimus,* whereas they are in tandem and slightly to one side of the body in *Prohemistomum.* Consequently, the author does not concur in the synonymy proposed by Mehra (1947).

The genus *Prohemistomum* is redefined as follows: with the characters of the family Cyathocotylidae, subfamily Prohemistominae (see Szidat, 1936). Adult small, body undivided, but with a short papilla evident in living specimens. Ventral concavity not pronounced, tribocytic organ in posterior half of body, weakly developed and with median fissure. Ventral sucker immediately anterior to tribocytic organ. Testes in tandem arrangement and slightly to one side. Cirrus sac well-developed. Vitellaria confined to posterior half of body. Cercaria with characters of the Vivax Group and Subgroup (Sewell, 1922), *i.e.,* with acetabular primordium, furcae with dorsal and ventral fin-folds, and three pair of flame cells in the tail stem. Includes: *P. vivax* (Sonsino, 1892) Azim, 1933, type; *P. odhneri* Mehra, 1947; *P. secundum* Vidyarthi, 1948; and *P. chandleri* sp. nov.

Of the described species of *Prohemistomum, P. chandleri* most closely resembles *P. secundum* in spination, in the size and shape of the oral sucker and pharynx, in the presence of a moderately long esophagus, and in the posterior position of the testes. In having very unequal suckers it differs from *P. secundum* in which the ventral sucker is nearly as large as the oral sucker, the tribocytic organ is more anterior in position, and the ovary is farther posterior than in *P. chandleri.*

P. vivax and *P. odhneri* can be distinguished from *P. chandleri* in having oral and ventral suckers of approximately the same size, lacking an esophagus, and having the tribocytic organ at a more anterior level.

THE GENUS HOLOSTEPHANUS

Although Cable and Vernberg (1949) reported an adult cyathocotylid from the channel catfish, *Ictalurus punctatus,* the naming and more complete description of this species has been deferred for inclusion in the present paper.

Holostephanus ictaluri sp. nov. (Figs. 5–7)

Specific diagnosis: with the characters of the genus *Holostephanus.* Body spinose, round or slightly ovoid in shape, length 1.02–1.61 (average, 1.42), maximum width 0.949–1.50 (average, 1.34). Oral sucker wider than long, measuring 0.88 × 0.131 to 0.160 × 0.182 (average, 0.127 × 0.143). Length of pharynx, 0.073–0.088 (average, 0.081). Ventral sucker 0.083 × 0.073 to 0.094 × 0.088 (average, 0.087 × 0.080) situated immediately posterior to pharynx, cup-shaped with opening directed posteriad toward tribocytic organ which may partially obscure it. Esophagus very short, ceca long, extending to or beyond level of cirrus sac. Tribocytic organ massive and well-developed, nearly filling the ventral concavity and in unflattened specimens, with a longitudinal slit-like opening. Testes irregular in shape, situated obliquely, the right anterior to the left. Posterior testis measures 0.226 × 0.146 to 0.306 × 0.219 (average, 0.257 × 0.208); anterior testis 0.168 × 0.124 to 0.210 × 0.153 (average, 0.190 × 0.132). Cirrus sac well-developed, measuring 0.234 × 0.117 to 0.299 × 0.131 (average, 0.275 × 0.127). Ovary oval, measuring 0.131 × 0.088 to 0.197 × 0.146 (average, 0.159 × 0.120), situated on left and anterior to level of anterior testis. Vitelline cells large, arranged in a corona encircling the tribocytic organ, but not meeting anteriorly. One to 11 eggs in uterus at one time and measuring from 0.109 × 0.088 to 0.117 × 0.102 (average, 0.114 × 0.094).

Host: Ictalurus punctatus (Rafinesque).

Site: Small intestine.

Locality: Wabash and Tippecanoe Rivers, Lafayette, Indiana.

Of 114 catfish which have been examined since *H. ictaluri* was first reported (Cable and Vernberg, 1949), five were infected with a total of nine specimens. This

incidence was extremely low in comparison with that of other parasites in the fish. This may have been due to very infrequent exposure to infection or persistence of the adult worm in the intestine of the fish for only a short time. Probably both factors were concerned, but certain observations indicate that the latter may have been the more important of the two. In natural infections with cyathocotylids which have been reported by other investigators, a low incidence is the general rule. Faust and Tang (1938) have suggested that these forms remain only a short time in the intestine due to the evolutionary trend of the group toward poorly developed adhesive organs so that these worms are unable to attach themselves firmly to the musosa.

H. ictaluri is only feebly attached to the intestine of the host and is easily dislodged. In fact, when a freshly killed fish is opened, the worms often are free in the lumen of the intestine. This may be associated with the tendency of the species to evert the tribocytic organ so that the margin of the ventral concavity is rolled dorsally and the dorsal surface of the worm is reversed in contour from convex to concave with the caudal papillae and oral sucker directed toward each other (Fig. 7).

The genus *Holostephanus* was erected in 1936 by Szidat, and placed in the subfamily CYATHOCOTYLINAE. He characterized the genus as having a rounded body with a ventral concavity almost entirely filled with the tribocytic organ. The genus originally included two species, *H. lühei,* the type, and *H. bursiformis.* In the same paper (1936) Szidat also proposed the genus *Cyathocotyloides,* distinguishing it from *Holostephanus* by the relative size of the tribocytic organ. He allocated two species to the genus *Cyathocotyloides, C. curonensis* (Szidat, 1933) and the new *C. dubius.* However, Yamaguti (1939) emphasized the extent to which the size of the tribocytic organ may vary and concluded that Szidat's separation of the genera *Cyathocotyloides* and *Holostephanus* was unwarranted. He accordingly reduced *Cyathocotyloides* to synonymy with *Holostephanus* and described two new species, *H. metorchis* and *H. nipponicus,* which occur in Japanese birds.

Mehra (1943) redefined the genus *Holostephanus,* described four new species from Indian birds, and transferred *Cyathocotyle calvusi* Verma, 1936, to that genus. This disposition of *C. calvusi* was made on the basis of its resemblance to *H. nipponicus* in the general shape of the body, the size ratio of the oral sucker and the pharynx, the size of the testes, the extent and shape of the cirrus sac, and host. It may be pointed out, however, that in *H. calvusi* the ovary lies postero-lateral to the anterior testis, while in all other described species of *Holostephanus* the ovary is either directly anterior to the anterior testis or at that level but toward the opposite (left) side of the body. Furthermore, no mention is made of the ventral concavity in *H. calvusi,* which distinguishes *Cyathocotyle* from *Holostephanus.* It would therefore seem unwarranted to transfer *Cyathocotyle calvusi* to *Holostephanus.* Mehra also removed two species described by Faust and Tang (1938) to this genus. In their original paper, these authors provisionally designated the two as *Linstowiella lutzi* and *L. bambusicolae,* presumably because an acetabulum was lacking. However, Mehra was of the opinion that this structure was merely feebly developed and hence overlooked. *Linstowiella lutzi* has several characteristics in common with the genus *Holostephanus,* the principal ones being the arrangement of the gonads and the distribution of the vitelline follicles. The testes are on opposite sides of the body and the ovary is pretesticular. *L. bambusicolae,* on the other hand, is

more like members of the genus *Linstowiella* as revised by Anderson and Cable (1950) and in the writer's opinion, should be returned to that genus with which it is in agreement in the arrangement of the testes, the position of the ovary, the absence of an acetabulum, and the distribution of the vitelline follicles.

Of the adult characteristics known for *Holostephanus,* the position of the gonads and the presence of a ventral concavity are the most consistent. In all of the described species, the testes are on opposite sides of the body and usually diagonal in arrangement. The ovary is always at a level anterior to that of the anterior testis and may be on the opposite side of the body. The ventral concavity is conspicuous in living specimens, but in fixed preparations it is not so prominent. Other characteristics which are common to members of the genus include the presence of a well-developed cirrus sac, and extent of the vitelline follices to the level of the ventral sucker or pharynx.

With this combination of characters as a criterion, the following species are here regarded as belonging to the genus *Holostephanus: H. lühei* Szidat, 1936; *H. bursiformis* Szidat, 1936; *H. curonensis* (Szidat, 1933); *H. dubius* Szidat, 1936; *H. lutzi* (Faust and Tang, 1938) Mehra, 1943; *H. nipponicus* Yamaguti, 1939; *H. metorchis* Yamaguti, 1939; *H. corvi* Mehra, 1943; *H. nephroni* Mehra, 1943; *H. anhingi* Mehra, 1943; *H. ibisi* Mehra, 1943; *H. thaparus* Vidyarthi, 1948; *H. phalacrocoraxus* Vidyarthi, 1948; and *H. ictaluri,* sp. nov.

Of these, *H. ictaluri* most closely resembles *H. curonensis.* The size ratio of the suckers, extent of the tribocytic organ, distribution of the vitelline follicles, the position of the ovary and the egg size are similar for the two species. They differ, however, in that *H. ictaluri* has a larger body size, the cirrus sac is more extensive and more muscular, the gonads are larger, and a fish instead of a bird serves as the definitive host.

DISCUSSION OF THE TAXONOMY OF THE FAMILY CYATHOCOTYLIDAE

Lutz (1935) outlined a taxonomic scheme for the family CYATHOCOTYLIDAE to include the species known at that time. He retained the subfamily CYATHOCOTYLINAE Mühling, 1898, to include a single genus *Cyathocotyle,* and proposed the subfamily PROHEMISTOMINAE for the genera *Prohemistomum, Mesostephanus,* and *Gogatea.* The two subfamilies were distinguished from each other by the shape of the body, which is elongate and bipartite (sic) in the subfamily PROHEMISTOMINAE, whereas it is rounded and undivided in the subfamily CYATHOCOTYLINAE.

In a comprehensive study of the family CYATHOCOTYLIDAE, Szidat (1936) proposed three new subfamilies, retaining PROHEMISTOMINAE and CYATHOCOTYLINAE, but recognizing four genera in the latter. In lacking a ventral concavity, two of these, *Cyathocotyle* and *Paracyathocotyle,* were separated from the others, *Holostephanus* and *Cyathocotyloides.* The genera *Paracyathocotyle* and *Cyathocotyle* were distinguished chiefly by the absence of a ventral sucker in *Paracyathocotyle.* However, Dubois (1938) reduced the latter to synonymy after finding that one of the species of *Paracyathocotyle* did have a ventral sucker. Szidat (1936) differentiated the genera *Holostephanus* and *Cyathocotyloides* on the basis of the relative size of the tribocytic organ, which, however, was considered invalid by Yamaguti (1939) in reducing *Cyathocotyloides* to synonymy with *Holostephanus.*

The subfamily PSEUDHEMISTOMINAE, erected by Szidat (1936) for a single

genus, *Pseudhemistomum,* was distinguished from members of the CYATHOCOTYLINAE primarily by its slightly bisegmented body. The writer observed that in *Holostephanus ictaluri* a papilla could often be observed in the living worms, giving them a somewhat bipartite appearance, and in a single whole mount this structure was distinct. In all other such material, however, there was no indication of the papilla, which actually is the strigeoid hind body, responsible for bisegmentation. Thus it is the writer's opinion that this characteristic is unreliable and does not justify the erection of the subfamily PSEUDHEMISTOMINAE. This view was stated by Mehra (1943).

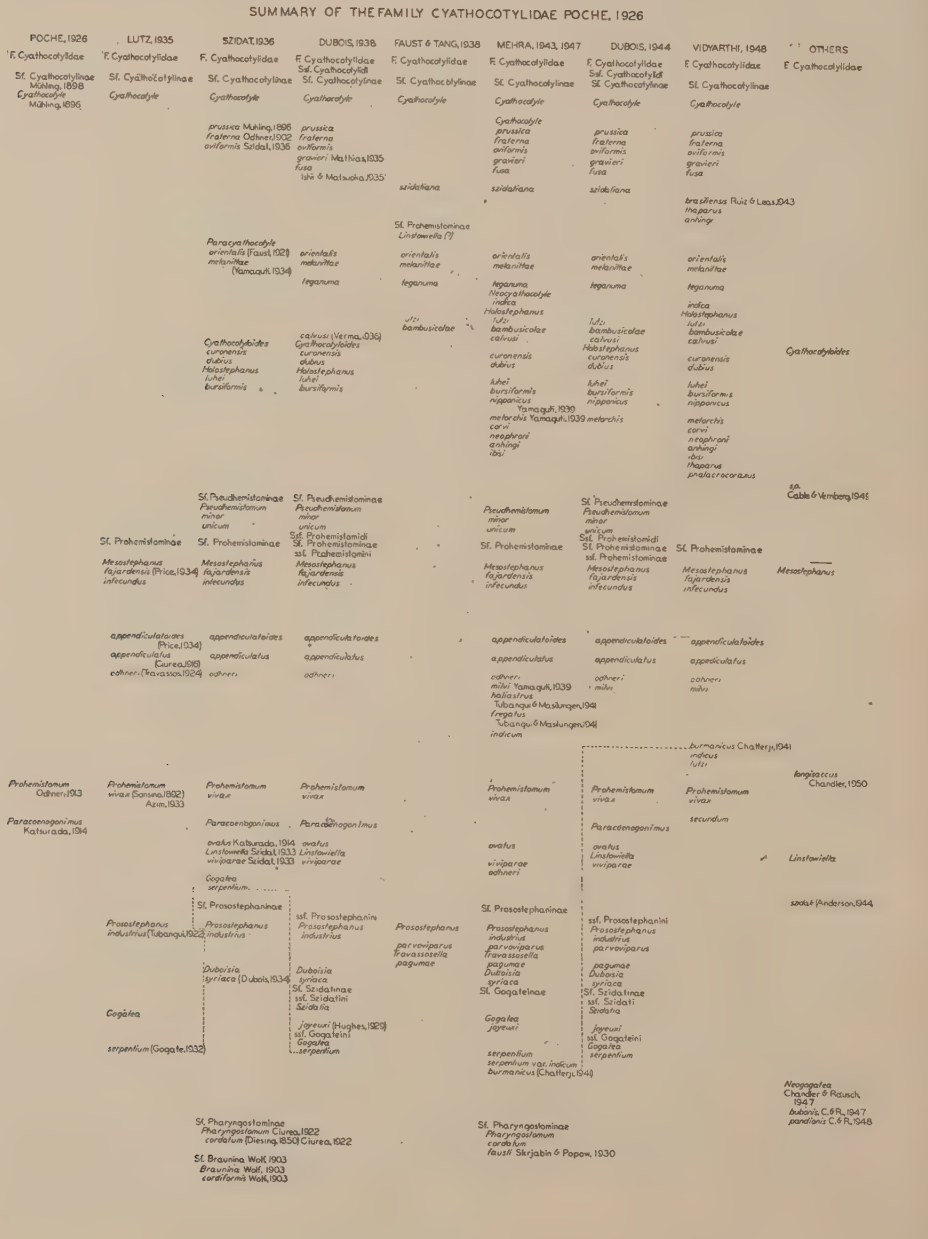
Szidat (1936) assigned the genera *Prosostephanus* and *Duboisia* to the new subfamily, PROSOSTEPHANINAE. In possessing a massive tribocytic organ and having the vitelline follicles confined to the area of that structure, this subfamily was distinguished from the PROHEMISTOMINAE. Szidat also proposed a new subfamily, the PHARYNGOSTOMINAE, to include the single genus *Pharyngostomum.* Dubois (1938) disagreed radically with this view and assigned the genus to another family, the DIPLOSTOMIDAE. However, Mehra (1947) returned it to the CYATHOCOLIDAE. Szidat also included the subfamily BRAUNINAE Wolf, 1903, in the family CYATHOCOTYLIDAE, although other workers have considered it to be a separate family which probably has evolved from the cyathocotylids.

Dubois (1938) proposed an elaborate scheme of classification for the strigeoids, using host specificity as a major taxonomic characteristic. He followed the scheme of Poche (1926) in reducing the families into many subdivisions. Later (1944) he elaborated further on his position regarding the importance of host specificity in the classification of the group. In doing so, he divided the family CYATHOCOTYLIDAE into two supersubfamilies, with the distribution of vitelline follicles as the chief distinguishing characteristic. In one, the CYATHOCOTYLIDI, the vitellaria extend up to the level of the pharynx but never invade the tribocytic organ. To this supersubfamily he allocated the subfamilies CYATHOCOTYLINAE and PSEUDHEMISTOMINAE, both of which are characterized also as being parasites of birds. However, two of the genera of subfamily CYATHOCOTYLINAE have species occurring in hosts other than birds. These are *Cyathocotyle fraterna* Odhner, 1902, and *C. brasiliensis* Ruiz and Leão, 1943, both from the crocodile. Furthermore, the present study reports *H. ictaluri* as a parasite occurring naturally in catfish. As more species are discovered, the number of such exceptions probably will increase.

The other supersubfamily, the PROHEMISTOMIDI, was characterized by Dubois as having vitelline follicles which are either confined posterior to the ventral sucker and arranged in two groups lateral to the tribocytic organ or penetrating that structure. Since there is much variation in the distribution of the vitelline follicles, not only does it seem that this character is of less value than has been ascribed to it, but the degree of intergradation makes it most difficult to utilize this character to separate major categories. Dubois separated the supersubfamily PROHEMISTOMIDI into two subfamilies, the PROHEMISTOMINAE and SZIDATINAE, mainly on the basis of definitive hosts, the PROHEMISTOMINAE being parasites of birds and mammals while the SZIDATINAE occur in reptiles.

The subfamily PROHEMISTOMINAE was further divided into two subsubfamilies, the PROHEMISTOMINI and the PROSOSTEPHANINI, which corresponded respectively to the subfamilies PROHEMISTOMINAE and PROSOSTEPHANINAE of Szidat (1936).

The distinguishing morphological characteristics of these subsubfamilies are quite apparent and it is the writer's opinion that they should not be placed in the same subfamily as Dubois has done, merely because both are parasites of birds and mammals. In the PROHEMISTOMINI, the tribocytic organ is small and the vitellaria are posterior to the ventral sucker, whereas in the PROSOSTEPHANINI there is a massive well-developed tribocytic organ and the vitelline follicles are confined to it.



The subfamily SZIDATINAE of Dubois is limited to those species occurring in reptiles. It also is divided into subsubfamilies, again based on the distribution of the vitellaria. In the subsubfamily SZIDATINI, the vitelline follicles are posterior to the ventral sucker and distributed in two separate lateral groups adjacent to the sides of the tribocytic organ. In the subsubfamily GOGATEINI, the follicles are arranged in a corona encircling the tribocytic organ. Each subsubfamily is represented by a single genus, the type.

Although Mehra (1947) supported Dubois in recognizing a separate subfamily for cyathocotylids of reptiles, he did not believe that further division into subsubfamilies was warranted. However, there is growing evidence that cyathocotylids occurring in similar hosts may not necessarily be more closely related than those utilizing dissimilar hosts. Chatterji (1940) reported *Mesostephanus burmanicus* occurring naturally in snakes in Burma, although other species of *Mesostephanus* are parasites of birds and mammals. Furthermore, Chandler and Rausch (1947) erected for a new cyathocotylid from owls the genus *Neogogatea,* which, as they state, is closely related to *Gogatea,* species of which occur in reptiles. Later, Chandler and Rausch (1948) recovered another species of *Neogogatea* from the intestine of an osprey. Finally may be cited from the present study the occurrence in fish of *Holostephanus ictaluri,* while other species in this genus are parasites of homoiothermal hosts.

In view of this lack of host specificity at the generic level, the writer is of the opinion that the type of host has been unduly emphasized as a taxonomic criterion of higher categories. In a group such as the CYATHOCOTYLIDAE in which the metacercaria develops far toward the size and structure of the adult and may require a very short time in a final host to become sexually mature, it is possible, and perhaps to be expected, that metacercariae could excyst and become sexually mature in several host species before their elimination from the intestine. In support of this view may be cited the fact that certain species will develop to maturity in a variety of hosts. Furthermore, the natural infections with cyathocotylids that have been reported have usually been extremely low in intensity and incidence, and, for that reason, the significance of the host type as an indication of relationships of the parasites is to be discounted.

SUMMARY

Holostephanus ictaluri, sp. nov. is described from the channel catfish, *Ictalurus punctatus* (Rafinesque), and the life cycle of *Prohemistomum chandleri,* sp. nov. is demonstrated experimentally. The cercaria of *P. chandleri* is of the Vivax group and subgroup; it develops in sporocysts in the digestive gland of *Pleurocera acuta* (Say), and encysts in the large- and small-mouth bass, *Huro salmoides* and *Micropterus dolomieu.* In feeding experiments with a variety of animals, living excysted worms were recovered from the intestine of the fishes, *I. punctatus* and *Cottus bairdii,* and the water snake, *Natrix sipedon.* No specimens contained eggs in the uterus, although several from *N. sipedon* were almost mature. The genera *Prohemistomum* and *Holostephanus* are redefined, and the taxonomy of the CYATHOCOTYLIDAE discussed, rejecting the type of definitive host as a basis for separating generic and suprageneric groups.

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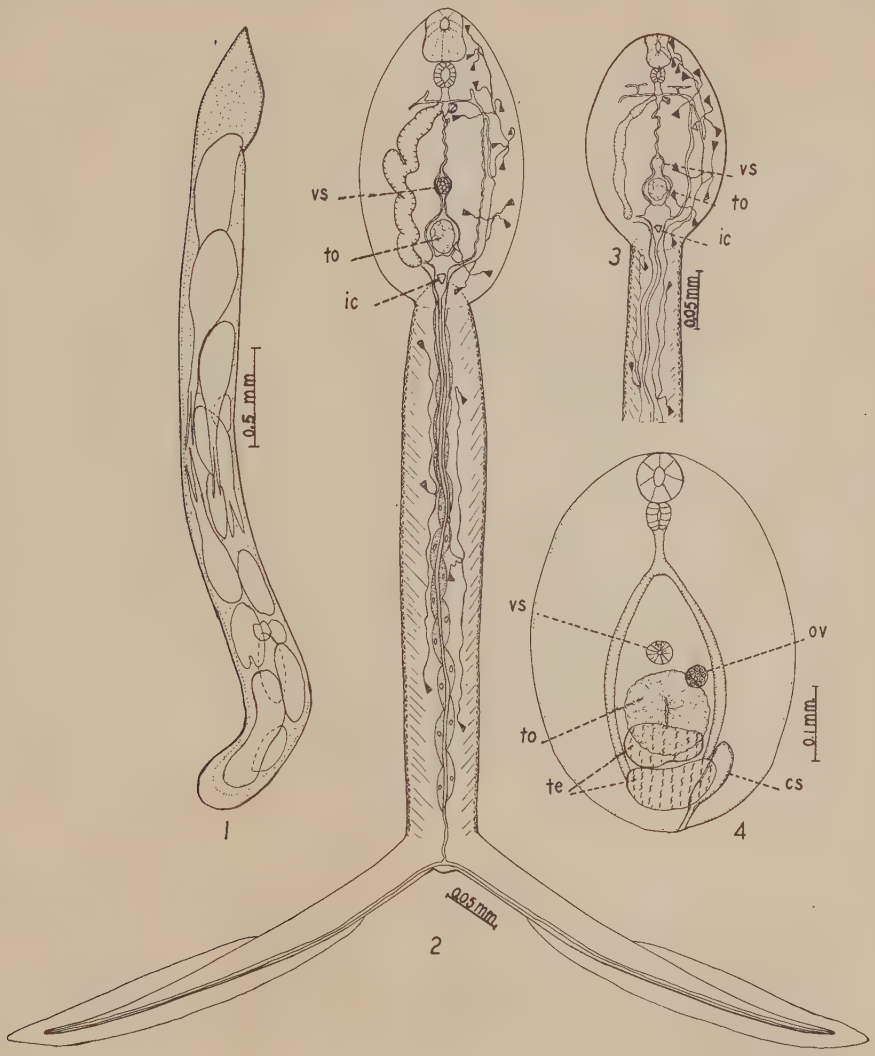

Plate I

Explanation of Plate I

Fig. 1. Sporocyst of *Prohemistomum chandleri* drawn by microprojection.
Fig. 2. Cercaria of *Prohemistomum chandleri,* free hand from living material.
Fig. 3. *Cercaria kentuckiensis,* excretory system free hand from living material.
Fig. 4. *Prohemistomum chandleri,* adult, ventral view of whole mount, drawn by microprojection.

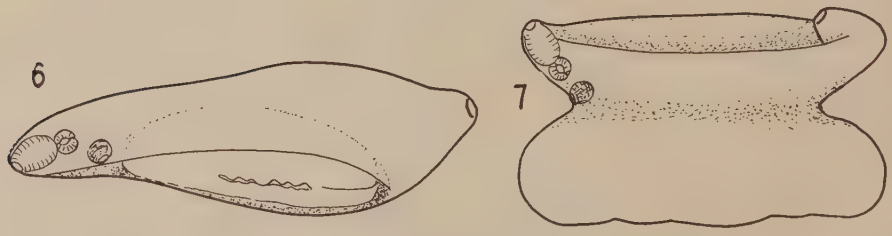

PLATE II

EXPLANATION OF PLATE II

(All figures concern *Holostephanus ictaluri*)

FIG. 5. Ventral view of whole mount, drawn by microprojection.

FIG. 6. Free hand sketch of living specimen in which the tribocytic organ is not everted.

FIG. 7. Same but with the tribocytic organ everted.

Lettering: *ci,* cirrus; *cs,* cirrus sac; *ic,* island of Cort; *ov,* ovary; *sv,* seminal vesicle; *te,* testis; *to,* tribocytic organ; *vs,* ventral sucker.

HELMINTHS OF NORTHWESTERN MAMMALS. PART II. *OLIGORCHIS NONARMATUS* N. SP. (CESTODA: HYMENOLEPIDIDAE) FROM THE YELLOW-BELLIED SQUIRREL

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During the past year, Mr. Clyde M. Senger, Department of Biology, Reed College, Portland, Oregon, and the author have had the opportunity to collect many interesting helminths from some of the more common northwestern mammals. As might be expected, a number of undescribed forms along with known species of parasites have been found. It is the purpose of this paper to report a new species of cestode of unusual taxonomic interest.

Two mature specimens of this tapeworm were recovered from the small intestine of a yellow-bellied squirrel, *Tamiasciuris d. douglasii* (Bachmann), collected near Eagle Creek, Oregon. The cestode was first tentatively identified as an anoplocephalid, primarily because it has an unarmed scolex and its egg was thought to have a pyriform apparatus. However, certain features of its anatomy made this identification uncertain; consequently, the material was sent for further study to Dr. Robert Rausch, Chief, Animal-borne Disease Branch, Arctic Health Research Center, Anchorage, Alaska, who has contributed much to the knowledge of North American rodent parasites. Dr. Rausch and his co-worker, Mr. Everett L. Schiller, placed the cestode in the family HYMENOLEPIDIDAE Fuhrmann, 1907, and upon further consideration of the problem, this identification appears to be well justified. Although the generic identity of this cestode has not been established with complete certainty, it seems advisable to present a description at this time so that it will be available for the consideration of other investigators. To the best of the author's knowledge, this cestode should be placed in the genus *Oligorchis* Fuhrmann, 1906, as emended by Fuhrmann, 1913. A complete discussion of the taxonomy of this cestode will be presented after its morphology has been considered.

The present study was made from whole mounts of two mature specimens of the cestode. One specimen was stained with Semichon's aceto-carmine and the other with Kornhauser's hematin. The stained material was cleared in terpineol and temporary wet mounts were found to be quite adequate for the study of the finer anatomy of this worm. This technique has been tried on a variety of smaller cestodes and has the advantages that it does not tend to make such material brittle, does not appreciably affect the quality of stained material stored in it for long periods, and allows the study of genital and excretory ducts which usually require serial sections. It is essential, however, that the terpineol be protected from moisture since it is quite hydroscopic and will pick up traces of water which will tend to make preparations cloudy and less desirable for study. All measurements are given in millimeters, except where otherwise stated, with average values included in parentheses. Drawings were prepared with the aid of a camera lucida.

I wish to take this opportunity to thank Dr. Robert Rausch and Mr. Everett L.

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Schiller for aid in identifying this parasite, Mr. Clyde M. Senger for help in collecting and preparing the material for study, and Dr. Ralph W. Macy for the use of the facilities of the Reed College Biology Laboratories.

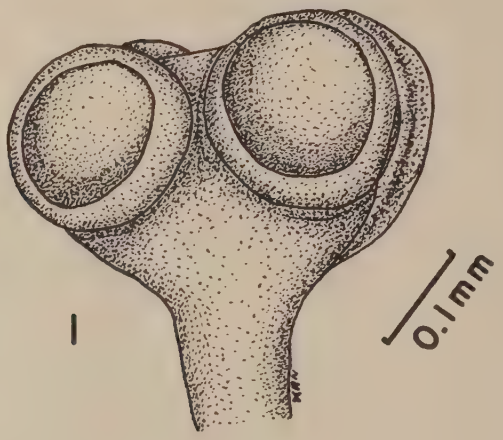

FIG. 1. Scolex.

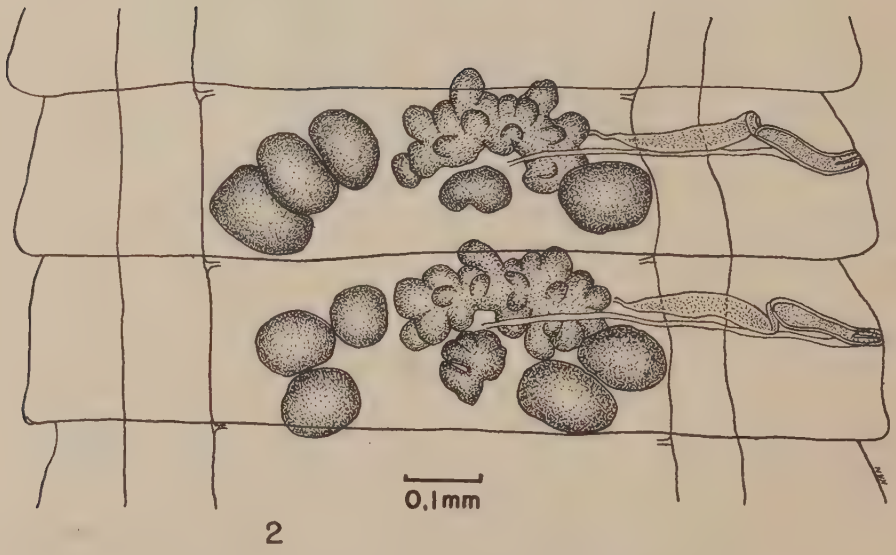

FIG. 2. Two consecutive proglottids showing two common types of testes arrangement.

Oligorchis nonarmatus n. sp. (Figs. 1, 2)

Diagnosis: Strobila margins serrate; scolex strongly developed; approximate lengths of two specimens 12 and 18 cm.; maximum width about 1.32. Suckers prominent, apparently not heavily muscled; they measure about 0.163 in diameter. The suckers constitute bulk of scolex, which is somewhat expanded in region just posterior to them. Rostellum absent. Proglottids all broader than long; mature segments about four times as broad as long. Genital pores dextral, medio-marginal, and unilateral (in one specimen, 3 out of about 840 proglottids have sinistral genital pores; in the other specimen, the genital pore was never found to be sinistral). In the

former specimen, other anomalous segments were also present. Large ventral and smaller dorsal longitudinal excretory canals run ventral to the genital ducts along both margins of strobila.

Genital organs similar to those of other hymenolepids. Cirrus pouch well developed, extending about two-thirds of way to excretory vessels; it measures 0.127–0.174 (0.146) long by 0.024–0.038 (0.033) broad, and is usually directed toward the anterior margin of proglottid. Cirrus unarmed. Internal and large external seminal vesicles present; latter about a fourth larger than cirrus pouch and extending to lateral margin of ovary. Four to six large, oval testes in each segment; average number is five, and one proglottid had only three. Testes usually situated three aporal and two poral within lateral margins of excretory vessels; in some segments there is one poral testis with three or four aporal. Vagina is narrow tube opening ventral to cirrus pouch, passing posterior to it into interovarial region. Seminal receptacle not observed. Ovary and vitelline gland are located slightly poral to middle of proglottid, with latter immediately posterior to former; ovary composed of many lobes. Uterus in gravid segments divided into numerous pockets, fills region of proglottid median to excretory vessels. Eggs broadly oval, measuring 0.038–0.045 (0.042) long by 0.031–0.036 (0.034) broad; hexacanth larva is 0.014–0.017 long by 0.012–0.014 broad.

Host: Tamiasciuris d. douglasii (Bachmann).

Habitat: Small intestine.

Locality: Eagle Creek, Clackamas County, Oregon.

Type: Slides bearing the two specimens collected have been deposited in the helminthological collection of the U. S. National Museum, slide number 47600.

DISCUSSION

In few other groups of cestodes is the number of testes as useful as a diagnostic character as it is in the HYMENOLEPIDIDAE Fuhrmann, 1907. Because of the constancy of testes number, hymenolepids with one, two, three, and four testes have been placed in separate genera. Other morphological characteristics, such as the course of the genital ducts, presence of an external seminal vesicle, position of the genital pore, and shape of the proglottids, have been of considerable use in hymenolepid taxonomy, but they appear to be less significant than testes number. The species dealt with in the present paper is considered a hymenolepid primarily because of the few large testes present, a unilateral genital pore, an external seminal vesicle, and short, broad proglottids. Because the testes are variable in number, it is difficult to assign this species to a particular genus with certainty. It is possible, however, to limit it to two previously described genera, and the real problem appears to be that of deciding whether or not one is a synonym of the other.

Oligorchis Fuhrmann (1906), as emended by Fuhrmann (1913), with 4–11 testes and *Pseudoligorchis* Johri (1934), emended by Johri (1941), with 4–12 testes are both possible genera for the reception of the present species. It can be readily seen that in respect to testes number the preceding genera overlap one another. However, since *Pseudoligorchis* differs from *Oligorchis* in the course of its genital ducts between the excretory canals, it is questionable whether it should not be considered a synonym of the latter genus.

Fuhrmann (1906) erected the genus *Oligorchis* for the reception of hymenolepids having 4 testes. Fuhrmann (1913) included *Oligorchis paucitesticulatus* with 7–11 testes in this genus primarily because it had an external seminal vesicle, and emended the genus to include hymenolepids with 4–11 testes. Mayhew (1926) has expressed the opinion that the presence of an external seminal vesicle, while almost universal amongst the members of the HYMENOLEPIDIDAE, does not seem to be sufficient grounds in itself for the inclusion of *Oligorchis paucitesticulatus* Fuhrmann, 1913, in that genus, and for that reason he listed the preceding species as a

doubtful species of *Oligorchis*. Mayhew diagnosed *Oligorchis* as having 4 testes, Joyeux and Baer (1936) for no apparent reason defined *Oligorchis* as hymenolepids with 4–7 testes. Southwell and Lake (1939) were in agreement with Joyeux and Baer (1936) when they described *Oligorchis kwangensis* with 4–7 testes.

Pseudoligorchis was erected by Johri (1934) and diagnosed as follows: "Scolex with an (?) unarmed rostellum. Genital pores unilateral. Genital ducts pass between the longitudinal excretory vessels. Testes numerous (more than 4) posterior and lateral to female glands. External *vesicula seminalis* present. Uterus an irregular lobed sac." He included *Oligorchis paucitesticulatus* Fuhrmann, 1913, with 7–11 testes and *Pseudoligorchis magnireceptaculata* Johri, 1934, with 8–12 testes in the genus. Johri (1941) emended *Pseudoligorchis* to include *Oligorchis kwangensis* Southwell and Lake, 1939, with 4—7 testes.

Apparently the main point of disagreement has been whether or not to include species with more than 4 testes in the genus *Oligorchis*. Fuhrmann (1913), Joyeux and Baer (1936), and Southwell and Lake (1939) are in favor of doing so, while Mayhew (1926) and Johri (1934, 1941) are not. This problem has come up in the classification of species belonging to other genera. According to Hughes (1941), *Hymenolepis acicula-sinuata* Rosseter, 1909, and *H. sibirica* (von Linstow, 1905) Fuhrmann, 1908, both have two testes instead of three which are characteristic of a large majority of the species of *Hymenolepis*. Rausch (1949) described *Paradilepis simoni* with five testes whereas the genus had previously been diagnosed as having 4 testes. Joyeux and Baer (1950) agreed with Rausch's diagnosis of *Paradilepis simoni*. Although it appears to be primarily a matter of opinion, it seems needlessly confusing to maintain a genus which is so nearly similar to another. As far as can be ascertained from the written descriptions, the only real point of difference between *Oligorchis* and *Pseudoligorchis* is that in the latter, the genital ducts pass between the excretory vessels instead of dorsal to them as in the former genus. It does not seem justifiable to maintain a genus on this basis alone and therefore it is the author's opinion that *Pseudoligorchis* should be considered a synonym of *Oligorchis*. Although it is undoubtedly very desirable to be able to diagnose taxonomic groups concisely without exceptional features, this can not always be accomplished. The variant forms which occur in natural populations do not readily lend themselves to this. On the other hand, the erection of new taxonomic groups to include forms which exhibit minor variations does not appear to be a very satisfactory solution of this problem.

If *Pseudoligorchis* is to be considered a synonym of *Oligorchis*, then *P. magnireceptaculata* Johri, 1934, *P. paucitesticulatus* (Fuhrmann, 1913), Johri, 1934, and *P. kwangensis* (Southwell and Lake, 1939) Johri, 1941, must be transferred to *Oligorchis*. The following key can be used to separate *Oligorchis nonarmatus* n. sp. and the other members of the genus.

Key to the Species of Oligorchis

1. Testes not variable in number in a strobila, numbering 4 in a proglottid 2
   Testes variable in number in a strobila, numbering 4–12 in a proglottid 5
2. Hooks number 50–52 ..... *O. yorkei* (Kotlan, 1923)
   Hooks number 22 or less 3
3. Hooks, 10 in a single row ..... *O. toxometra* Joyeux, Gendre, and Baer, 1928
   Hooks, 14–18 in number 4

Hooks, 20 in a single row ..... *O. longivaginosus* Mayhew, 1926
Hooks, 22 in a double row ..... *O. burmanensis* Johri, 1941
4. Hooks, 14–16 in a single row, 0.034 long ..... *O. strangulatus* Fuhrmann, 1906
Hooks, 16–18 in a double row, 0.083–0.100 and 0.113–0.190 long .... *O. hierticos* Johri, 1934
5. Scolex unarmed 6
Scolex armed 7
6. Rostellum present, 8–12 testes ..... *O. magnireceptaculata* (Johri, 1934)
Rostellum not present, 4–6 testes ..... *O. nonarmatus* n. sp.
7. Testes number 4–7; 10 hooks, 0.012 long ..... *O. kwangensis* Southwell and Lake, 1939
Testes number 7–11; 10 hooks, 0.016–0.018 long ..... *O. paucitesticulatus* Fuhrmann, 1913

SUMMARY

Oligorchis nonarmatus n.sp. from the yellow-bellied squirrel, *Tamiasciuris d. douglasii* (Bachmann), is described. The genus *Pseudoligorchis* Johri, 1934, is considered a synonym of *Oligorchis* Fuhrmann, 1906, as emended by Fuhrmann, 1913. A key to the species of *Oligorchis* is presented.

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STUDIES ON THE LIFE CYCLE AND BIOLOGY OF *MONOECOCESTUS SIGMODONTIS* (CESTODA: ANOPLOCEPHALIDAE) FROM THE COTTON RAT, *SIGMODON HISPIDUS*

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Since Stunkard (1937) worked out the first anoplocephalid life cycle on *Moniezia expansa,* the life cycles of 10 other members of the typical subfamily, ANOPLOCEPHALINAE, representing 7 genera, have been described. This work, except that pertaining to *Monoecocestus americanus* and *M. variabilis,* which was subsequently reported by Freeman (1949, 1952), has been summarized by Kates and Runkel (1948).

In each case mites of the superfamily ORIBATOIDEA, exclusively, have been shown to be the intermediate hosts. The mites involved belong to several different families and many genera, showing little specificity other than that resulting from ecological factors.

In addition to the life cycles mentioned above, Rendtorff (1948) obtained development of the cysticercoids of *Oochoristica ratti* in larvae and adults of COLEOPTERA and LEPIDOPTERA, but not in mites of the genus *Galumna.* This, together with other characteristics in which the genus *Oochoristica* and other members of the subfamily LINSTOWIINAE differ from the typical anoplocephalids, throws some doubt on the propriety of including them in the family. The eggs lack a pyriform apparatus, the uterus breaks up into egg capsules, and the hosts are arthropod-eating vertebrates, including reptiles.

The life cycle of a twelfth species of ANOPLOCEPHALIDAE, *Monoecocestus sigmodontis* (Chandler and Suttles, 1922) is described in this paper. This worm is a common and widely distributed parasite of cotton rats, *Sigmodon hispidus,* having been reported in about 65% to 75% of these animals in localities in southern United States from Texas to the Atlantic coast. The original specific diagnosis should be emended to include minute spines, about 2.5 μ long, on the outer shells of the eggs, similar to those described by Freeman (1949) on the outer shells of the eggs of *M. americanus* and *M. variabilis* (Fig. 1). Such spines have not been reported from other species of the genus, although Vigueras (1943) described and figured mammilations on the eggs of *M. gundlachi* which are totally unlike the spines here described. However, the spines are not visible on eggs in the uterus and are usually absent from eggs from feces or preserved specimens.

MATERIALS AND METHODS

Collections of mites were made from cotton rat runways in an attempt to secure natural infections and to determine the most prevalent species having access to the rats and to the eggs of the cestode. Mites for experimental purposes were collected from areas where there was no evidence of the presence of cotton rats. Periodic

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examinations of mites from both sources were made, but no natural infections were ever found in many hundreds of mites.

The mites were isolated from grass, roots, and top soil by means of a modified Berlese funnel, described by Kates and Runkel (1948). Using a 100-watt bulb in a white reflector, suspended approximately 3 inches above the top of about 4 inches of material, the mites were collected after 16 hours, separated according to species under a dissecting misroscope, and placed on moistened bits of filter paper in half-pint jars closed by screw-cap lids. Grass and soil heated sufficiently to destroy predators (80° C. for 30 to 40 minutes) were added to the jars to a depth of 1 to 2 inches. Molds and bacteria were not all killed by this heating and could serve as food for the mites. No excessive growth of molds such as Freeman (1950) encountered was evident, possibly because of antagonistic action between the larger number of different micro-organisms left in the cultures as compared with Freeman's culture material, which was heated to 180° C. for 2 hours. Transfer of mites to new cultures from time to time was therefore not necessary. The cultures were kept at room temperature, which varied from 24° C. to 26° C. during the winter months, and from 28° C. to 32° C. during the summer months, or in an incubator at 26° C. to 28° C. with a high humidity. They were kept moist to a point just below saturation by the addition of water to the jars when required. Cultures of the smaller species such as *Protoschelobates seghettii* and *Oribatula minuta* were successfully maintained by these methods, but as found by other authors (Krull, 1939b; Freeman, 1950) species of *Galumna* were most difficult to maintain.

Eggs of *Monoecocestus sigmodontis,* collected from feces of infected rats by centrifugal flotation, from gravid proglottids obtained at autopsy, or from shed proglottids on the surface of the fecal pellets, suspended in water, were added to filter paper and placed in the jars with freshly collected mites, and left there from three to five days before the addition of the grass and soil.

For examination for developing larvae, 5 to 10 mites at a time were removed from the cultures, placed in a drop of saline, and cracked under a cover glass by gentle pressure, which did not injure the cysticercoids. Uninfected, laboratory-reared cotton rats were exposed to infection by feeding infected mites suspended in saline, either with a pipette or by means of a stomach tube. Infected mites used for experimental infections were always cracked before being fed to the rats, since preliminary experiments confirmed Freeman's observation that no infections resulted from feeding intact mites. This observation would indicate that in nature the mites must be broken by the normal chewing of the food.

Feces from the experimentally infected animals were checked daily by simple smears and by direct centrifugal flotation, beginning about 2 weeks after infection.

RESULTS

1. *Experiments on Eggs.* When unbroken proglottids, commonly passed in the feces on the surface of the pellets, were placed on a slide and left at room temperature in a petri dish, it was found that the eggs are forcibly ejected from the uterus as the segment dries and contracts. After 10 days the eggs were found free of the proglottid at distances of about 1.5 mm. to 3.0 mm. This same phenomenon was reported by Hall (1934) as occurring in *Thysanosoma actinioides* and by Stunkard (1938b) in *Moniezia expansa* and *Cittotaenia.* Stunkard concluded that

such ejection of eggs increases the probability of their being ingested by a mite, and prevents mass infections which might be fatal.

To determine the duration of viability, eggs from gravid proglottids were mixed with feces, placed in petri dishes, some covered and some uncovered, and left at room temperature (28° C. to 30° C.) to dry as they normally would in nature. The onchospheres remained viable and active for 2 weeks, but were dead after 3 weeks. There was no difference in the duration of viability of the eggs from covered and uncovered petri dishes. Eggs from gravid proglottids, free of feces, contained active larvae after being kept in water in a refrigerator at 5°C for 3 months. Krull (1939a) reported that the eggs of *Cittotaenia* survive for as long as 325 days under similar conditions.

Egg production was studied in single-worm infections produced by feeding one cysticercoid to each rat. Beginning shortly before the end of the prepatent period, twenty-four hour outputs of feces were collected daily, thoroughly comminuted, diluted to 100 cc. with tap water, and 0.1 cc. samples counted, each egg representing 1000 eggs in the original feces. The egg production rose steadily after the onset (55 to 58 days after infection) finally levelling off in about 4 weeks at a remarkably constant output of 13,000 to 14,000 eggs per day.

Counts on gravid proglottids gave an egg content ranging from 4,000 to 5,000 eggs in proglottids from the surface of the fecal pellets, and from 7,000 to 7,500 in proglottids from worms recovered at autopsy. Apparently the proglottids from feces had lost some of the eggs before being collected. A careful search of the feces never revealed more than 2 proglottids in a 24-hour period, and in view of the daily egg production and the egg content of the segments, it is probable that this is the normal number of proglottids shed daily.

2. *The Intermediate Hosts.* Since oribatid mites have been shown to be intermediate hosts for all species of ANOPLOCEPHALINAE for which the life cycle is known, these mites were exposed to experimental infections by the methods described above. In addition, however, direct infection of the cotton rat was attempted by feeding ripened eggs by stomach tube, but with negative results. Also, about 50 *Tenebrio molitor* were exposed to infection on two different occasions with completely negative results, although the beetles remained alive for over 65 days.

The mites collected and identified were found to belong to five families and nine species as follows, the numbers following the species names indicating the approximate numbers examined: ORIBATULIDAE, *Protoschelobates seghettii* (4000) and *Oribatula minuta* (1000); BELBIDAE, *Belba* sp. (1000); LIACARIDAE, *Liacarus* sp. (700); GALUMNATIDAE,[2] *Galumna minutum* (200), *G. banksi* (2000), *G. emarginatum* (2000), and *G. virginiense* (300); and HAPLOZETIDAE, *Rostrozetes* sp. (200). Complete development of cysticercoids was obtained in five of these nine

[2] The name of this family is based on the genus *Galumna*, erected in 1828 by von Heyden for certain species of Latreille's genus *Oribata*. Galumna is apparently a misspelling of the Greek neuter noun Galumma, meaning a head covering, and must then be considered a neuter noun, and the family name should be Galumnatidae instead of Galumnidae, which has hitherto-fore been used by all writers. The adjectives used for specific names in the genus *Galumna* have been written with masculine, feminine, and neuter endings, *e.g.*, *G. minutus*, *G. alata*, *G. virginiensis*, and *G. emarginatum*. Not only different authors, but even individual authors, have been inconsistent in their interpretations of the gender, often using the genders of the genera in which the species were originally described. Von Heyden made *Notaspis alatus* the type species, but failed to make the new combination.

species: *P. seghettii,* 9%; *O. minuta,* 1%; *Belba* sp., 2%; *Liacarus* sp., 1%; and *G. minutum,* 0.5%. *P. seghettii* (Fig. 6), which had the highest rate of infection, also harbored the largest average number of cysticercoids per infected mite (8), and was the most common species in the collections from the rat runways. This mite has previously been reported by Runkel and Kates (1947) from Beltsville, Maryland, as a vector for *Moniezia expansa* with an infection rate of 6%. *P. seghettii* plays the same role as chief vector for *M. sigmodontis* in the Houston area that *Liacarus itascensis* does for *M. americanus* and *M. variabilis* in Minnesota (Freeman, 1952). The lowest rate of infection occurred in *Galumna minutum,* in which a single specimen out of 200 examined was found infected. No infections were found in the three other species of *Galumna* represented in the collections, although large numbers were examined. Partial development to the vermiform stage occurred in *G. banksi,* but although hundreds of mites of this species were dissected at later periods after exposure to infection, no mature cysticercoids were found. This refactoriness of species of *Galumna* to development of *M. sigmodontis* is in contrast to the important part played by mites of this genus as intermediate hosts for *Moniezia expansa.* Two species of *Galumna* were found by Freeman (1952) to serve as hosts for *M. americanus* and *M. variabilis* also. The species of *Belba* and *Liacarus* could not be positively identified and may be new species.

In all, 3.5% of experimentally infected mites developed mature cysticercoids, averaging 7 per mite. The highest number of cysticercoids in individuals was 13, occurring in a specimen of *Belba* sp. and in *P. seghettii,* and the lowest number, 4, occurring in *Galumna minutum* and *Oribatula minuta.*

3. *Development in the Intermediate Host.* Actual ingestion of the eggs by the mites in laboratory cultures has not been observed, so the exact time of infection is not known. Krull (1939b) observed that the mouth parts of the mites were too small to ingest the entire egg, and that a hole was made in the outer membranes and the contents ingested. In our cultures at least of the smaller species, empty egg shells have been observed, so it is possible that only the embryo and pyriform apparatus are ingested.

In cultures kept at room temperature (24° C. to 26° C.), a free larval form, 0.019 mm. in diameter, and coarsely granular in appearance, was recovered from the body cavity of a mite (*G. banksi*) one week after exposure to infection. Only 5 of the 6 hooks were visible. At the end of two and one-half weeks, forms were recovered in this same species and also in *P. seghettii* which had grown to a diameter of 0.035 mm. to 0.04 mm. (Fig. 2). These were coarsely granular, showing numerous large rounded cells, and containing a vacuole about one-third the width of the larva in diameter. Only 2 to 4 hooks were seen in these forms. After four weeks, elongated forms were found in *G. banksi* which measured 0.115 by 0.046 to 0.065 mm., the vermiform stage (Fig. 3). These were wider at the anterior end with a definite indication of suckers; the vacuole observed in the earlier forms was no longer in evidence. Another larval form was recovered from *G. banksi* with slightly more developed rudiments of suckers. This larva was more oval than the other, measuring 0.080 by 0.072 mm.

Developmental forms similar to those described above were found at about the same ages in *P. seghettii* which were kept in an incubator at 26° C. to 28° C. However, the four-week-old larvae were somewhat more advanced than those recovered

from *G. banksi* kept at 24° C. to 26° C.; these larvae are pre-invaginated or segmented forms, having an over-all length of 0.214 mm. and a maximum diameter of 0.070 mm. The body is divided by two constrictions into two more or less spherical anterior portions and a more slender posterior portion. The anterior portion, which is the developing scolex, measures 0.050 to 0.060 mm. by 0.060 to 0.070 mm.; it is granular in appearance, with a thin wall, and developing suckers. The middle portion, 0.065 to 0.070 mm. in diameter, is muscular with a thicker body wall, and is the cystic portion into which the scolex is later withdrawn. The posterior part, 0.100 mm. in length, is the cercomere (Fig. 4).

At the end of five to six weeks the scolex has already invaginated. The cyst wall had closed over in some of the forms recovered at this stage, but in many the invagination canal could still be seen. At this stage the suckers are well formed, but have not yet reached their full development. The cercomere has shortened to a conical structure about 0.050 to 0.060 mm. long, half its length in the preceding stage.

During the next week or two there is further development of the suckers, and a thickening of the cyst wall (Fig. 5). The cercomere shrinks further in diameter, usually persisting, even in mature cysticercoids, as a small tail-like appendage about 0.050 mm. or less in length. Mature forms recovered at the end of eight weeks have a rather fibrous outer wall and an active, well-developed scolex which almost completely fills the inner cavity of the cyst. The diameter of the cysticercoids is about 0.1 mm.; of the scolex 0.080 mm., and of the suckers 0.030 mm. Calcareous granules are present.

The rate of development was affected only slightly by the temperatures employed (24° C. to 32° C.), and crowding of the larval forms in the mites did not appear to affect the rate of development or size. Cysticercoids from mites in which 10 or 12 of them completely filled the body cavity were as large or only slightly smaller than those from mites containing only 4 or 5.

It will be seen that the development of *M. sigmodontis* in the intermediate host closely parallels that of other species of Anoplocephalinae. Freeman (1950, 1952) reported in detail the morphology of various phases in the development of *M. americanus* and *M. variabilis* in the porcupine. The principal differences noted between these species and *M. sigmodontis* is the greater degree of development of the suckers before invagination of the scolex takes place, and the much larger cercomere in the infective cysticercoids in the porcupine species. In *Cittotaenia* the suckers are rudimentary when the scolex is invaginated, and the cercomere shrinks to a very small appendage on the mature infective cysticercoid (Stunkard, 1941). In *Moniezia expansa,* also, the cercomere is shrunkened to a small fibrous appendage (Stunkard, 1938a). The appearance of the cercomere in *M. sigmodontis* more nearly resembles that of *Moniezia expansa* and *Cittotaenia.* Other differences in development of different species of anoplocephalids are in size of the mature cysticercoids and the time required for their development. *Monoecocestus americanus* matures in 45 days at 25° C., reaching a diameter of 0.137 mm., whereas *M. variabilis* requires 52 days at 20° C. (diameter 0.152 mm.) and 81 days at 15° C. (diameter 0.170 mm.), according to Freeman (1952). According to Stunkard (1937), *Moniezia expansa* at room temperature required 105 to 112 days, reaching a diameter of 0.18 mm., although Anantaraman (1951) reported full development in 5

weeks during the warm season in India, and in 7 weeks during the cold. *Cittotaenia* spp. require 70 to 120 days, reaching a diameter of 0.17 mm. to 0.2 mm. (Stunkard, 1941), and *B. studeri,* 56 days, reaching a diameter of 0.11 mm. (Stunkard, 1940). Potemkina (1944a, 1944b) reported that *M. benedeni* matures in 100 to 120 days at 26° C., and in 140 to 150 days at 17° C. to 19° C., and *T. giardi* developed in 120 to 160 days at 17° C. to 19° C. Sizes of the mature cysticercoids were not given. Anantaraman (1951), however, reported development of *M. benedeni* in 6 to 8 weeks.

4. *Development in the Definitive Host.* To complete the life cycle, laboratory-bred cotton rats were fed the mature cysticercoids by the methods already described.

One animal fed cracked *P. seghettii* containing 6 cysticercoids, was autopsied 6 weeks later and found to harbor 2 immature worms. Another similarly-fed animal remained negative over a period of 3 months. Each of 5 other cotton rats was fed from 10 to 15 cysticercoids from *P. seghettii.* Eight weeks later eggs and proglottids were recovered from the feces of all five. Subsequent experimental infections have confirmed these results, that approximately 8 weeks are required for the cysticercoids to develop into adult worms.

Since Stoll (1937) reported that *Moniezia expansa* could not be found in the sheep intestine up to the eighth day after infection, and suggested that a parenteral phase of development may take place before the worms are found in the lumen, attempts were made to locate early stages of *Monoecocestus sigmodontis* either in the mucosa or lumen of the small intestine of the cotton rat. Three animals, fed 40 to 50 cysticercoids, were autopsied 72 hours later. The gut was tied off and cut into 4 sections, and the lumen of each flushed out with a stream of warm water. The washings were carefully examined. The gut was then slit open and the entire surface of the intact mucosa examined under a microscope. The mucosa was then scraped and the villi thoroughly searched microscopically. Although searched for hours by two microscopists, no evidence of worms could be found either in the lumen or in the tissue. No conclusions can be drawn regarding the presence or absence of tissue stages without further investigations. Control animals fed at the same time became infected, indicating that the cysticercoids used were infective.

At the end of one week after infection, young worms were recovered from the lumen of the gut. These measured 0.85 mm. to 1.8 mm. long by 0.2 mm. wide. The scolex was 0.230 mm. in diameter with suckers 0.060 to 0.070 mm. in diameter. After two weeks, the worms measured 5.0 to 7.0 mm. long and 0.86 mm. wide, with scolices 0.330 mm. in diameter and suckers 0.110 to 0.120 mm. in diameter. Four-week-old worms were 15 to 18 mm. in length by 2.0 mm. wide, the scolices measuring 0.340 mm. and the suckers 0.110 to 0.115 mm. in diameter. The major portion of the growth of the worms had been attained by the fourth week, as those obtained after six and eight weeks were only slightly larger. The six-weeks-old worms measured 25 to 32 mm. long by 1.5 to 2.5 mm. wide, with a scolex of 0.340 to 0.380 mm. and suckers 0.110 to 0.130 mm. in diameter. At eight weeks, when shedding of ripe segments began, they were 30 to 60 mm. in length by 2.5 to 3.0 mm. in width with the scolex 0.400 to 0.450 mm. and the suckers 0.130 to 0.160 mm. in diameter.

From observations on naturally-infected, wild trapped animals the length of life of *M. sigmodontis* in the definitive host is well over a year, since infected animals kept for as long as 15 months without opportunity for reinfection continued to shed

eggs and proglottids in the feces during the entire time, and at autopsy contained worms with gravid proglottids.

The percentage of worms established in experimental animals ranged from about 6% to 30%. Differences may be due to 1) immaturity of the cysticercoids even though they appeared mature, 2) individual variation in susceptibility of animals, and 3) time of feeding with reference to retention in the stomach. Cotton rats are primarily nocturnal, and since the experimental feedings were done in the afternoon, it is possible that the stomach may have been empty at this time. Although the mites containing the cysticercoids were cracked, it may be that a considerable time in the stomach, or stimulation of the flow of bile, is required to get the cysticercoids to evaginate and attach.

5. *Age Resistance.* No age resistance to *M. sigmodontis* was observed either in nature or in the laboratory, and in no instance were uninfected wild rats, of unknown age, found refractory to experimental infections.

To test this further, 2 rats that were 12 months old, and 2 that were 6 weeks old, were infected with 14 to 16 cysticercoids each. At autopsy, 8 weeks later, 1 old rat harbored 4 mature worms measuring, respectively, 1.45 cm., 2.48 cm., 3.4 cm., and 4.4 cm. (average 2.93 cm.); and the other had 2 worms measuring 3.7 cm. and 3.1 cm. The young rats harbored 2 worms measuring 4.5 cm. and 4.5 cm., and 3.5 cm. and 3.8 cm., respectively.

Although observations indicate that in nature an age resistance to other anoplocephalid infections exists, Stoll (1935) has shown that age is not a factor in resistance of sheep to *Moniezia expansa*, since two-year-old ewes (reared cestode-free) became infected with the same rapidity as eleven-week-old lambs.

6. *Resistance to Superinfection.* To determine whether there is resistance to superinfection, four groups of cotton rats, approximately eight weeks old, with four rats in each group, were given cysticercoids by stomach tube as follows:

The animals in Groups I-A and I-B were given 10 to 15 cysticercoids each. Group I-A received a second dose of the same number of cysticercoids eight weeks later, and a third sixteen weeks later (eight weeks after the second). Group I-B received a similar dose sixteen weeks after the initial inoculation. The animals in Groups II-A and II-B were given 30 to 40 cysticercoids, with Group II-A being given a second dose of the same number at the end of eight weeks and a third at the end of sixteen weeks, and Group II-B being given a similar dose at the end of sixteen weeks. All four groups were autopsied four weeks after the last dose, twenty weeks after the start of the experiment.

Two previously uninoculated control animals were given 10 to 15 cysticercoids at the time of the first re-inoculations, and two others were given the same number of cysticercoids at the time of the second re-inoculations. The controls were autopsied at the same time as the experimental animals.

Autopsies four weeks after the last inoculation would make it possible to distinguish the worms of the last inoculation from the older ones, which should have been mature, and comparison with the four-week-old worms in Control Group II would indicate possible effects of the presence of older worms on the growth and development of younger ones.

The results were as follows: The four rats of Group I-A, receiving three doses of 10 to 15 cysticercoids each, were each found to harbor a single worm, measuring

from 6.5 cm. to 7.5 cm., and no immature worms. Those of Group I-B, receiving two doses of 10 to 15 cysticercoids each, harbored 2, 2, 3, and 0 worms, respectively. The sizes ranged from 3.5 cm. to 4.5 cm. No immature worms were found. In Group II-A, receiving three doses of 30 to 40 cysticercoids each, 2 rats were found to have 3 and 4 worms, respectively, ranging in size from 2.5 cm. to 4.2 cm., and no immature worms. Two of the four rats in this group died before the experiment was completed. The last group, II-B, receiving two doses of 30 to 40 cysticercoids each, were found to have 5, 3, 3, and 3 mature worms, respectively, ranging in size from 2.5 cm. to 4.2 cm. Again, no immature worms were found. The two control rats infected 8 weeks prior to autopsy harbored 2 and 3 worms, respectively, measuring 2.7 cm. to 3.5 cm., and the two control animals fed 4 weeks prior to autopsy harbored 5 and 4 immature worms, respectively, measuring 1.5 cm. to 1.8 cm.

Absence of any immature worms in any of the 14 experimental animals that survived and were autopsied, indicates complete resistance to reinfection. In Group I-A, all of the animals were shedding eggs and proglottids eight weeks after the initial inoculation; and since at autopsy only a single worm was found in each, it is evident that the primary infection produced resistance to both subsequent inoculations. In Group II-A, 2 of which died before the conclusion of the experiment, the possibility exists that some of the worms may have developed from secondary inoculations, but in view of the few worms recovered, and the results in the three other groups, it is more probable that those recovered at autopsy developed from the initial inoculation. The viability of the cysticercoids in the second and third inoculations is indicated by the presence of worms in the two control groups.

From these limited but uniformly consistent data, it would seem that the presence of even a single worm may be sufficient to prevent establishment of a secondary infection, even after as short a period as eight weeks, which is the time required for the worms to become mature and being shedding eggs and proglottids. There is no evidence as to whether the resistance to superinfection is environmental or immunological in nature, but the smaller size of the worms in rats harboring several worms as compared with those in single-worm infections suggests the possibility that it may be due entirely to a crowding effect.

SUMMARY

The complete life cycle of *Monoecocestus sigmodontis* has been studied. Development of the cysticercoids occurs in the body cavity of species of Oribatoidea; it is completed in approximately 8 weeks. *Protoschelobates seghettii*, the most prevalent mite in the area studied, was the most efficient vector.

The cysticercoids mature in about 8 weeks. They are about 0.1 mm. in diameter, with a small tail-like cercomere, and a well-developed but small scolex, which is only 0.080 mm. in diameter as compared with 0.400 to 0.450 mm. in the adult worms. Infection of the definitive host, *Sigmodon hispidus*, occurs only when the infected mites are cracked before being fed. The prepatent period is approximately 8 weeks. In single-worm infections the egg production reaches a peak of about 13,000 to 14,000 eggs per day in about 4 weeks representing two shed proglottids per day. The infections may persist for more than 15 months. Comparisons of the life cycle with that of the other anoplocephalids have been made. No age resistance

was demonstrated, but there was evidence of an apparent resistance to superinfection, even when only single worms were present.

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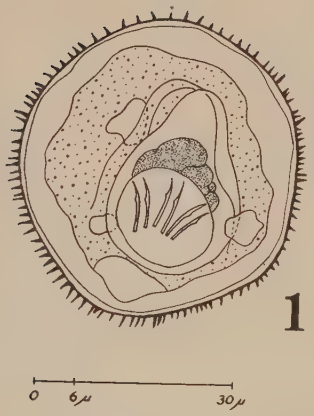
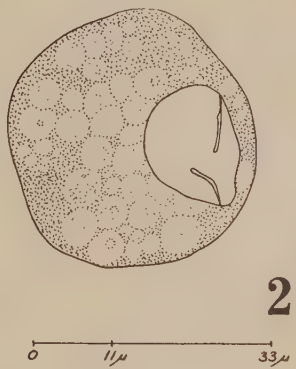
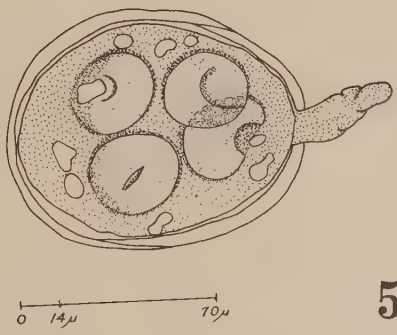
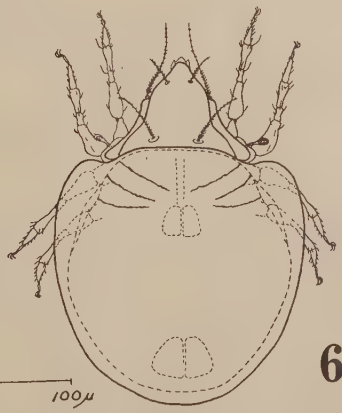
PLATE I









ANOTHER ANNELID FIRST INTERMEDIATE HOST OF A DIGENETIC TREMATODE

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Digenetic trematodes generally use mollusks as their first intermediate hosts. However, Linton (1915) discovered that an annelid, which he thought was *Hydroides dianthus* Verrill, serves in this capacity in the vicinity of Woods Hole, Massachusetts. Dr. Olga Hartman of the Hancock Foundation, University of Southern California, has informed me that the annelid collected in the Woods Hole region and called *Hydroides dianthus* or *H. hexagonus* by various authors is really *Eupomatus dianthus* (Verrill) Mörch, 1863. The larval trematode parasitizing that annelid was not named by Linton but Stunkard (1929) designated it *Cercaria loossi.* Martin (1944) added to the description of *C. loossi* and on a morphological basis suggested that it probably was a fish blood-fluke. Rankin (1946) examined a number of annelids collected in the Woods Hole region but found only *Eupomatus dianthus* infected and then only with *Cercaria loossi.*

It is the purpose of this paper to report another annelid, *Lanicides vayssierei* (Gravier), capable of serving as the first intermediate host of a digenetic trematode. Twelve specimens of *L. vayssierei* were dredged at 58 fathoms on January 29, 1948, off Cape Boyds, Ross Island, Antarctica, by the United States Navy Antarctic Expedition of 1947–48. These annelids were sent to Dr. Hartman for identification. She noted that one of them appeared abnormally swollen and presented the material to me for study. When the body wall of the annelid was slit open, a large number of elongate bodies emerged which proved to be rediae containing germ balls and, in a few instances, cercariae. The remaining 11 annelids were not infected. They had been preserved in formalin since the time of collection. Since no cercariae were found free in the coelom of the infected annelid, some were carefully dissected out of rediae, stained, and mounted. Whole mounts were also made of rediae.

OBSERVATIONS

Cercaria hartmanae n. sp.

(This species is named in honor of Dr. Olga Hartman. The type specimen is deposited as number 37366 in the U.S. Nat. Mus. A paratype is deposited in the Hancock Parasitology Collection number 482).

The cercariae, having been fixed within the rediae, were for the most part contorted and poorly extended. Also because living material was not available, some of the anatomy, such as that of the excretory system, could not be determined. The following measurements are based upon 24 whole mounts. The body length varies from 0.099 to 0.143 (average 0.124 mm.) and maximum body width, near the level of the acetabulum, from 0.028 to 0.037 (average 0.031 mm.). Cuticular spines are present in transverse rows at the anterior end of the body. The anterior organ is oval and measures 0.028 to 0.037 (average 0.031 mm.) in length and 0.015 to 0.022

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(average 0.019 mm.) in width. The acetabulum is located near the mid-level of the body and measures 0.012 to 0.015 mm. in diameter. A pharynx and eyespots are lacking. Five penetration glands are present on each side of the body. A small, almost spherical mass of cells staining more heavily than surrounding tissues is located near the posterior end of body. This is believed to be the genital primordium. A small V-shaped excretory vesicle was seen in some specimens. The cylindrical tail stem exceeds the body length, measuring 0.133 to 0.195 (average 0.158 mm.) and having a maximum width of 0.012 to 0.015 (average 0.013 mm.). The short caudal furcae measure 0.015 to 0.028 (average 0.023 mm.) in length and 0.006 mm. in width. They are devoid of fins.

Redia: The 52 rediae that were measured could be divided into two groups on the basis of size. Whether or not these represented different redial generations could not be determined but it is suggested by the heaviness of the infection. Individuals in the smaller-sized group varied from 0.294 to 0.434 (average 0.356 mm.) in length and 0.028 to 0.056 (average 0.046 mm.) in width. The smallest of this group was filled with cells except for the space occupied by a pharynx and tubular gut (Fig. 1) which extended posteriorly one-third to nearly one-half the length of the body. The pharynx size was essentially the same regardless of the size of the redia. Its length varied from 0.019 to 0.040 (average 0.030 mm.) and its width varied from 0.012 to 0.028 (average 0.019 mm.). Rediae of the larger-size group measured from 0.434 to 0.938 (average 0.63 mm.) in length and 0.098 to 0.21 (average 0.13 mm.) in width. The majority of this group were filled with germ balls and only the largest rediae contained cercariae.

DISCUSSION

Lanicides vayssierei (Gravier) is a tubiculous annelid which has been collected at many localities in the antarctic region: near Alexander Land and Port Circoncision (Gravier, 1911); Grahams Land, South Georgia, Kaiser Wilhelm II Land (Hessle, 1917); Alexander Land, Port Circoncision, Cape Adare, McMurdo Sound (Benham, 1921, 1927); Adelie Land, MacRobertsons' Land and South Georgia (Monroe, 1930, 1939). It has been taken at depths of from 3 or 4 to 350 fathoms. Whether or not it is infected with *Cercaria hartmanae* over its entire range remains to be seen. Gravier (1911) collected *L. vayssierei* at a little over 100 fathoms on January 16, 1909 and recorded the water temperature as 1.6° C. This was during the antarctic summer and it is very unlikely that the water temperature at this latitude and at such depths gets much above 1.6° C. Water temperatures in other comparable localities in the antarctic probably are very similar and thus it is evident that the development of some digenetic trematodes can proceed at low temperatures.

Lanicides vayssierei is a member of the family TEREBELLIDAE while *Eupomatus dianthus* (Verrill), host to *Cercaria loossi*, belongs to the family SERPULIDAE. It is striking that although *C. loossi* develops in sporocysts and *C. hartmanae* in rediae, both are apharyngeate, brevi-furcate cercariae. Martin (1944) stated that the anatomy of *C. loossi* suggested that its adult stage might be a blood fluke of some marine fish. *C. hartmanae* also resembles blood fluke cercariae in being apharyngeate, having a forked tail with short furcae, and possessing an anterior organ, instead of the muscular oral sucker typical of most cercariae.

SUMMARY

A new species of larval trematode, *Cercaria hartmanae*, is described. It develops in rediae in the coelom of a marine annelid, *Lanicides vayssierei* (Gravier), collected at 58 fathoms off Cape Boyds, Ross Island, Antarctica. *L. vayssierei* is a member of the family TEREBELLIDAE whereas the only other annelid known to act as first intermediate host of a digenetic trematode is *Eupomatus dianthus* (Verrill) belonging to the family SERPULIDAE. From this report it is evident that the larval stages of some digenetic trematodes may develop at near freezing temperatures. The anatomy of *C. hartmanae* suggests that its adult may be a blood fluke of some marine fish.

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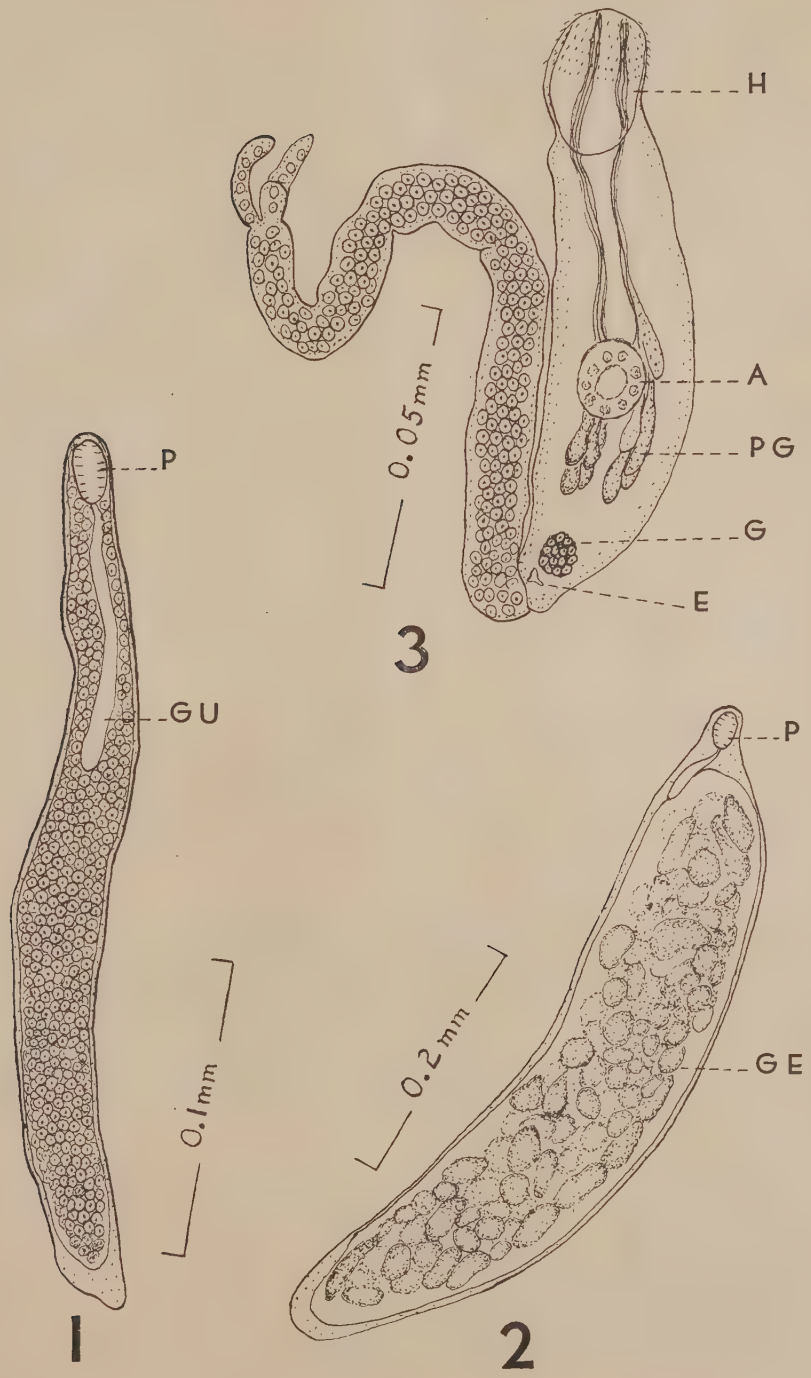
EXPLANATION OF PLATE

(All drawings made with aid of camera lucida)
All figures concern *Cercaria hartmanae*
FIG. 1. Small redia. FIG. 2. Large redia. FIG. 3. Cercaria.

Abbreviations used: A, acetabulum; E, excretory vesicle; G, genital primordium; GE, germ balls; GU, gut; H, anterior organ; P, pharynx; PG, penetration glands.


H
A
PG
G
E
0.05mm
3
P
GU
0.1mm
1
P
GE
0.2mm
2

COLLECTIONS OF ECTOPARASITIC MITES FROM ALASKA

H. L. KEEGAN[1] AND R. A. HEDEEN[2]

This report on ectoparasitic mites from Alaska is presented to supplement the few published accounts of mites from the territory (see references). All species concerned are members of the family LAELAPTIDAE. Specimens reported were obtained from three sources: Rocky Mountain Laboratory of the United States Public Health Service, through Mr. Glen M. Kohls; United States National Museum, through Dr. E. W. Baker; and Arctic Aeromedical Laboratory of the United States Air Force, through Captain John M. Geary. Among the specimens sent by Mr. Kohls were three collections made on Nunivak Island by personnel of the Arctic Health Research Center of the United States Public Health Service at Anchorage. Unmounted specimens were prepared for study by enlisted personnel of the survey section, 498th Preventive Medicine Company, Fort Sam Houston, Texas.

Specimens reported in this paper were taken from rodents and birds at several widely separated localities from Nunivak Island off the coast of southwestern Alaska to Umiat and Barter Island far above the Arctic circle. Eight species are represented in these collections. In the list which follows, Rocky Mountain Laboratory is abbreviated as RML, and Arctic Health Research Center is abbreviated as AHRC. Identified specimens have been returned to the institutions from which they were obtained.

Specific Determinations

Laelaps multispinosus Banks, 1909

3 females, 1 nymph from male muskrat, Summit Lake, 4 June 1951, J. M. Geary.

Laelaps kochi Oudemans, 1936

2 females from *Microtus* sp., Umiat, 18 June 1947, L. A. Jachowski.

Laelaps alaskensis Grant, 1947

4 females, 4 males, 3 nymphs from *Lemmus trimucronatus haroldi* Swarth., Nunivak Island, August 1951, Bert Babero, AHRC No. 10188; 3 females, 2 males AHRC No. 10202, data as for 10188; 2 females from 4 brown lemmings, Barter Island, 27 June 1951, G. M. Kohls, RML No. 29205; 2 females, 1 nymph from 4 brown lemmings (dead in trap), Barter Island, 27 June 1951, G. M. Kohls, RML No. 29210; 1 female from 3 brown lemmings (dead in cages), Barter Island, 1 July 1951, G. M. Kohls, RML No. 29214; 1 female from bank swallows nests, Chena River, 3 July 1951, G. M. Kohls, RML No. 29217.

Haemogamasus alaskensis Ewing, 1925

3 females from *Lemmus trimucronatus haroldi* Swarth., Nunivak Island, August 1951, Bert Babero, AHRC No. 10138; 23 females, 1 nymph from bank swal-

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lows nests, Big Delta, 20 June 1951, C. B. Philip, RML No. 28984; 2 females from tree swallow nest, Olnes, 21 June 1951, G. M. Kohls, RML No. 29204; 1 female from 4 brown lemmings, Barter Island, 27 June 1951, G. M. Kohls, RML No. 29205; 47 females from 4 brown lemming nests, Barter Island, 27 June 1951, G. M. Kohls, RML No. 29209; 1 female from lemming nest containing 9 young, Barter Island, 29 June 1951, G. M. Kohls, RML No. 29211; 1 female from 3 *Microtus* sp. (dead in traps), Barter Island, 30 June 1951, G. M. Kohls, RML No. 29213; 17 females, 1 male from bank swallows nests, Chena River, 3 July 1951, G. M. Kohls, RML No. 29217; 2 females from *Microtus* sp., Umiat, 6 June 1951.

Haemogamasus ambulans (Thorell, 1872)

2 females from 3 *Microtus* sp. dead in traps, Barter Island, 30 June 1951, G. M. Kohls, RML No. 29213; 1 female from *Microtus,* Chena River, 7 December 1950, J. M. Geary.

Haemolaelaps glasgowi (Ewing, 1925)

5 females from *Citellus,* Black Rapids, 21 June 1951, C. B. Philip, RML No. 28991.

Hirstionyssus isabellinus (Oudemans, 1913)

12 females, 2 males, 1 nymph from *Lemmus trimucronatus haroldi* Swarth., Nunivak Island, August 1951, Bert Babero, AHRC No. 10188; 2 females, 1 male, collecting data as in 10188, AHRC No. 10202; 1 female from *Microtus,* Umiat, 24 June 1947, L. A. Jachowski; 1 female from *Microtus,* Umiat, 25 June 1947, L. A. Jachowski; 4 females from *Microtus* sp., Umiat, L. A. Jachowski, 6 June 1947; 37 females from *Microtus* sp. #1, Umiat, 6 June 1947, L. A. Jachowski; 1 female from *Microtus* sp. #10, 24 June 1947, L. A. Jachowski; 1 female from *Microtus,* Big Delta, 19 June 1951, C. B. Philip, RML No. 28967.

Dermanyssus gallinae (Degeer, 1778)

22 females, 2 males from swallow nest, Gulkana, 9 August 1951, W. L. Jellison, RML No. 29823.

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THE COMPARATIVE TISSUE REACTION OF TWO STRAINS OF *AUSTRALORBIS GLABRATUS* TO INFECTION WITH *SCHISTOSOMA MANSONI*

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The importance of a knowledge of intermediate host-parasite relationships in the understanding of the epidemiology and prospects for control of a parasitic disease has long been recognized. The complexities of these relationships were recently emphasized for schistosomiasis, a disease afflicting millions throughout tropical and subtropical areas, by the studies of Files and Cram (1949) and Files (1951). Among other relationships, it was found that, whereas a Puerto Rican strain of a snail intermediate host, *Australorbis glabratus,* was quite susceptible to infection with a Puerto Rican strain of *Schistosoma mansoni,* a strain of this species of snail from São Salvador, Bahia, Brazil was highly refractory to infection.

In further studies with these strains, the writer has been investigating the influence of genetic and other factors in determining the susceptibility of the intermediate host. In the course of these investigations, observations were made on the fate of the parasite in the two strains of *A. glabratus.* It had been observed that the larval stage of the parasite, the miracidium, penetrated both the susceptible and nonsusceptible snail strains with apparently equal willingness and ease. However, aside from the fact that none of the developmental stages characteristic of the parasite when in the susceptible strain could be found in the Brazilian strain after the usual 4- to 5-week developmental period, nothing was known of the fate of the miracidia which had penetrated. Information relative to this question has been obtained from a comparison of the tissue response, after exposure to infection, of snails from both strains.

EXPERIMENTAL

Young adult members of both strains were exposed simultaneously to 30 to 50 miracidia per snail. At various intervals of time thereafter, infected snails of each strain were placed in water overlaid with menthol crystals. The latter procedure was adapted from Berry (1943). Apparently through an anesthetic or narcotic action, this treatment causes the snails to assume and remain in an extended position. Fifteen to 18 hours later, the extended snails were placed into Bouin's fixative. When fixed without previous menthol treatment the snails retract into their shells and provide poor sections.

After several hours in the fixative, most of the shell had dissolved. The anterior portion of the snail, including such areas as the head, foot, tentacles, and anterior mantle (i.e., the areas usually attacked by miracidia) was then severed from the rest

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of the body, dehydrated in graded alcohols, sectioned, and stained with haematoxylin and eosin. Sections were cut 6 micra in thickness and approximately 150 to 200 sections were examined for each snail. It was estimated that approximately two-thirds of the anterior portion of each snail was examined microscopically.

Two such series of sections were made. The first series included nine snails each of the susceptible Puerto Rican strain and the nonsusceptible Brazilian strain. In this series, snails were narcotized and fixed at daily intervals after exposure to infection. In the second series, sections were made of snails with infections of no more than 45 hours' duration. Included were 3 Puerto Rican and 10 Brazilian snails which had been exposed to infection, and a total of 19 snails of both strains which had not been exposed.

RESULTS

Sections of snails obtained in the first series revealed a striking difference in the fate of the miracidia. In the Puerto Rican strain, each snail contained several parasites which appeared to be developing normally without any obvious reaction on the part of the snail. There were considerably fewer parasites found than were used for exposing the snail. (It is a common finding that all the miracidia to which a snail is exposed do not penetrate.) Fig. 1 of Plate I represents a 42-hour infection (including time spent in mentholated water) in a Puerto Rican snail. In Fig. 2 there is shown a 6-day infection in another Puerto Rican snail of this series. Developing daughter sporocysts are present in various sections of the mother sporocyst. It is apparent that there was no obvious reaction to the parasite. These findings agree with those of Maldonado and Acosta-Matienzo (1947) who found no signs of cellular reaction to the parasite, in a study of the development of Puerto Rican *S. mansoni* in Puerto Rican *A. glabratus*.

In addition to the above usual findings in snails of the susceptible strain, there were a few areas of cellular infiltration such as would be indicative of a response to a foreign body. However, the stimuli for these responses were no longer present, or were not recognizable. Fig. 3 shows such an area in a Puerto Rican snail with a 4-day infection; this snail also contained normally developing parasites against which no reaction was apparent.

The results obtained with the nonsusceptible Brazilian strain were quite in contrast to those mentioned above. With but one exception, in none of the sections of the first series could any recognizable schistosome material be found. There were, however, in each snail, several areas of cellular reaction to unrecognizable stimuli. These areas were characterized by variably, but often rather concentrically, arranged layers of cells about a core. In some instances there appeared to be a fibrous type of tissue interwoven among these cells. In the snails fixed a few days after exposure these cellular aggregates were relatively easy to find; among those fixed at later intervals after exposure, it was often difficult to find any evidence suggestive of infection. Fig. 4 shows a typical finding in the Brazilian strain. In the one exception mentioned above, a single miracidium was found in the 2-day infection. There was no apparent cellular reaction to the parasite. However, development did not appear to be normal when compared with the parasites found in the Puerto Rican snail after 2 days.

While it was felt that the cellular aggregates found in the Brazilian snail were probably reactions to miracidia, the failure to find visible proof indicated the need

for a second series of sections consisting of younger infections. It was hoped that remnants of schistosome material could be found in at least some of the reaction sites.

In the second series, the results with the Puerto Rican snails paralleled those of the first. Normally developing parasites without cellular involvement were the rule. However, again there were a few cellular aggregates, the stimuli for which were not apparent.

In the Brazilian snails of the second series, no parasites without a reaction thereto were found. The majority of findings were, again, areas of cellular reaction to an inapparent stimulus. In addition, however, there were a few such areas which included remnants of miracidia. (Figs. 5 and 6.) These findings indicated that the reaction to the parasite in the Brazilian strain was a very rapid one, with destruction and phagocytosis of the parasite usually accomplished within 24 to 48 hours.

The question arose regarding whether or not the few areas of reaction found in the susceptible strain were responses to the presence of miracidia. The findings with the unexposed control snails suggested a possible cause for these areas.

In 5 unexposed Puerto Rican snails taken from the same aquarium as were those which were exposed to schistosome infection, a total of 10 such areas were found. The type of reaction appeared to be similar to the few areas encountered in the Puerto Rican snails exposed to infection. The stimuli for the areas in the control snails were not apparent but presumably were responses to some other infecting organism present in the aquarium. Consequently, it is quite likely that the few areas of reaction found in the susceptible snails along with normally developing parasites may actually have been present prior to the time the snails were exposed to infection.

In 14 unexposed control Brazilian snails, no areas of cellular reactions were found. This suggested that the infecting organism present in the Puerto Rican snail aquarium was not present in the Brazilian snail aquarium. Consequently, it is quite probable that most, if not all, the cellular aggregates found in this strain after exposure to infection represented responses to miracidia.

The comparison between the strains is further emphasized by Table 1. In this table are included the findings with the 1- and 2-day infections of the first series and all snails of the second series. Numerical comparisons of the findings in the snails with older infections in the first series are not included. In the susceptible strain, individual enumeration of the parasites was difficult in view of their large size and tortuous shape; in the nonsusceptible snails it was difficult to find any evidence of infection. The findings are divided into three categories: 1) Parasites against which there was no apparent cellular response, 2) sites of cellular reaction but in which obvious remnants of schistosome material were present, 3) cell agglomerates within which the stimulating agent was not present or recognizable.

It can be noted from the table that of 38 items found in 5 snails of the susceptible Puerto Rican strain after exposure, 34 were apparently normal miracidia. The other 4 findings were areas of reaction, the excitatory agents of which were not apparent, and which might have been present prior to exposure to infection with miracidia.

In contrast, of 90 items found in 12 nonsusceptible snails, 74 were areas of cellular reaction without recognizable stimuli. There were 15 other areas of cellular reaction, but in these remnants of schistosome material could be found. In only one

instance was a miracidium found, around which there was no cellular reaction. However, as indicated earlier, this organism did not appear to be normal.

SUMMARY AND CONCLUSIONS

A study of sections of 50 snails of a Puerto Rican and a Brazilian strain of *Australorbis glabratus* revealed differences which account, in part, for the failure to infect the latter strain with a Puerto Rican strain of *Schistosoma mansoni.* In the susceptible Puerto Rican strain, the parasite develops apparently without any effective interference on the part of the snail. In contrast, in the Brazilian strain the parasite is destroyed and removed, usually within 24 to 48 hours after penetration. As at least a part of this response, there is a marked cellular infiltration around the parasite. A fibrotic type of walling-off reaction also appears to be characteristic of this response.

TABLE 1.—*Comparative Findings in Sections of Puerto Rican and Brazilian* Australorbis glabratus *After Exposure to Puerto Rican* Schistosoma mansoni

Snail strain and number	Age of infection at fixation (hours)	Number of miracidia without cellular reaction	Number of areas of cellular reaction	
			With schistosome material	Without recognizable stimuli
Exposed to Infection				
Puerto Rican				
1.	21	5	0	0
2.	24	3	0	1
3.	42	7	0	0
4.	45	6	0	2
5.	66	13	0	1
Totals	..	34	0	4
Brazilian				
1.	21	0	2	5
2.	21	0	3	6
3.	21	0	1	7
4.	24	0	3	3
5.	24	0	0	3
6.	24	0	0	6
7.	42	0	0	11
8.	45	0	2	12
9.	45	0	2	7
10.	45	0	1	4
11.	45	0	1	8
12.	66	1	0	2
Totals	..	1	15	74
Not exposed to infection				
Puerto Rican				
1.	..	..	..	3
2.	..	..	..	0
3.	..	..	..	5
4.	..	..	..	0
5.	..	..	..	2
Totals	..	..	..	10
Brazilian				
1. to 14.	..	..	..	0

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PLATE I

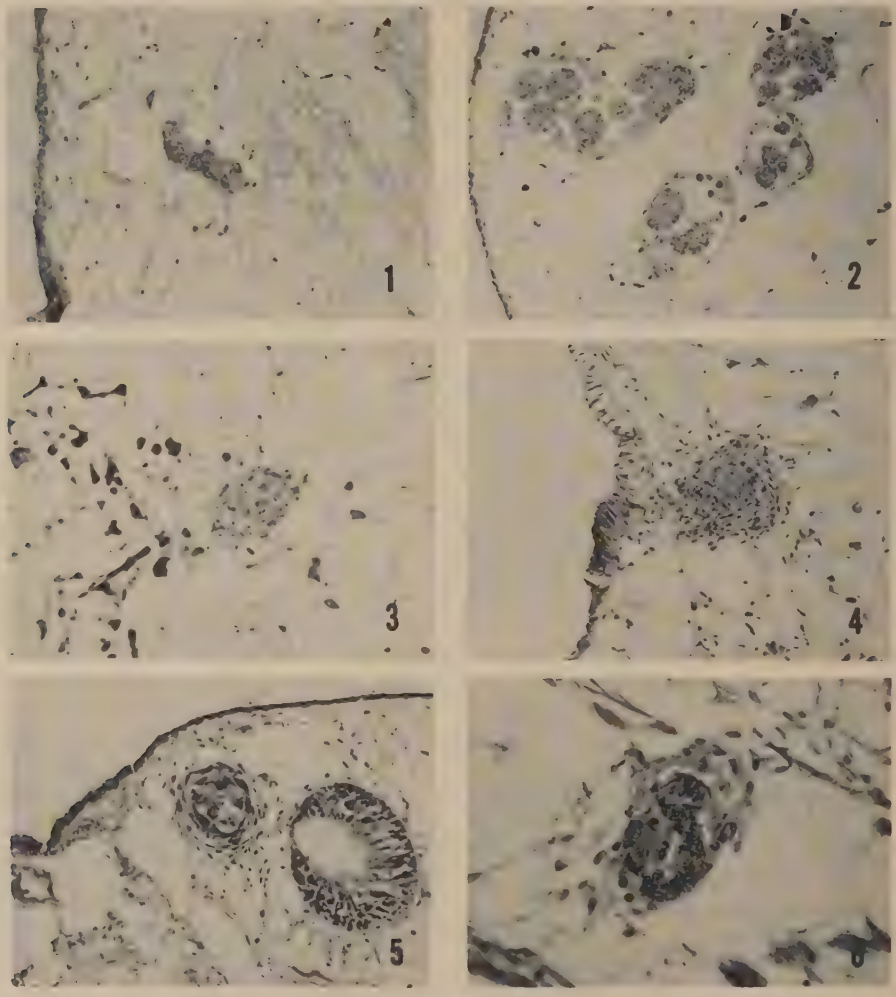

PLATE I. Sections of Puerto Rican and Brazilian *Australorbis glabratus* after exposure to Puerto Rican *Schistosoma mansoni*.

FIG. 1. 42-hour infection in Puerto Rican snail, ×247.
FIG. 2. 6-day infection in Puerto Rican snail, ×375.
FIG. 3. Cellular area in Puerto Rican snail with otherwise normal 4-day infection, ×150.
FIG. 4. Typical finding in Brazilian snail, 42 hours, ×375.
FIG. 5. Cellular area containing parasite in Brazilian snail, 21 hours, ×375.
FIG. 6. Parasite in Brazilian snail, 45 hours, ×494.

RESEARCH NOTES

THE DISCOVERY OF TRICHINA CYSTS IN THE DIAPHRAGM OF A SIX WEEK-OLD CHILD

During a trichinosis survey recently carried out in Vancouver, British Columbia (Bourns, unpublished), the discovery was made of 22 trichina cysts per gram of muscle in the diaphragm of a six week-old child. Early writers believed that intrauterine infection did not take place. However, Roth (1935; Act. path. Scand. Kopenh.,**12:** 203–215 and 1936; Zent. Bakt. **136:** 278–284; cited in Gould, 1945, Charles C. Thomas, publs.) demonstrated muscle trichinae in the foetuses of guinea pigs infected during pregnancy, and Kuitunen—Ekbaum (1941, Can. Pub. Health Jour. **32:** 569–573) reported finding four live larvae in 6.5 grams of diaphragm tissue from a seven month-old foetus. Dr. L. E. Ranta (personal communication) described the finding of a heavy infection of trichinae in a foetus at autopsy in Toronto.

In the present case, the baby was born five weeks prematurely in a frail condition, and died six weeks later. The writer contacted the physicians who had attended the confinement and the child's illness, and was informed that the mother, a 17 year-old Canadian woman had suffered no illness during pregnancy.

The condition of the cysts is worthy of note inasmuch as the larvae were dead or absent, and many of the cyst walls showed calcification. Ehrhardt (quoted by Gould) observed in 1896, death of muscle larvae as early as the third week, and larval calcification between the 42nd and 60th days. Similarly Leuckart (in Gould) demonstrated calcification as early as the 80th day. Nonetheless, the process of calcification does not usually commence until after the sixth month of infection, and the writer feels that this case may indicate a difference in the rate of calcification in foetus from that in the adult.—T. K. R. Bourns, *Livestock Insect Laboratory, Kamloops, B.C., formerly with Department of Zoology, University of B. C.*

IS *HYMENOLEPIS CALIFORNICUS* (YOUNG, 1950) A SYNONYM OF *HYMENOLEPIS* (*WARDIUM*) *FRYEI* (MAYHEW, 1925)?

In a recent issue of this journal Schiller (1951; 266–272) has expressed the opinion that these two species are identical, basing his opinion largely on the similarity of the hooks.

Through the courtesy of Dr. E. W. Price of the U. S. Bureau of Animal Industry I have been permitted to examine Mayhew's specimen (U. S. B. A. I., #51289) and I find that Schiller's statement is correct. Mayhew's (Ill. Biol. Monogr. **10:** 1–125) drawing (fig. 27) is incorrect as he evidently overlooked the handle which is hard to see, due to the overlapping of the hooks.

Neither Schiller or Mayhew mention the musculature of *H. fryei* which in *H. californicus* is a distinctive feature. I have not had any cross sections of the former species available for examination, but in the whole mount the inner longitudinal muscles appear as numerous scattered strands, not grouped in definite bundles, and the same arrangement appears in Mayhew's figures, while in the latter they are grouped in four (dorsal and ventral) bundles, as shown in figures 2 and 4 of my paper (Young, 1950 J. Parasit. **36:** 9–12).

While the hooks are similar in the two species, the difference in the musculature confirms my belief that *Hymenolepis californicus* is a valid species and not a synonym of *H. fryei*.—R. T. Young, *San Diego, California.*

TRANSPLANTATION OF ADULT HEARTWORMS, *DIROFILARIA IMMITIS*, IN DOGS

During the course of an investigation of the treatment of canine filariasis with various compounds we attempted to transplant a definite number of adult filarids from infected (both treated and untreated) dogs into microfilaria-negative dogs with the following purposes in mind: 1) evaluation of any possible sterilizing effect of a drug upon the adult worms found alive in the treated animals at autopsy 2) obtaining a quantitative estimation of the number of microfilaria produced for a definite period of time by a single fertilized female worm.

With the exception of the negative results obtained by Wharton (1946, Science **104:** 30) in an attempt to transplant live adult *Litomosoides carinii* from an infected cotton rat, *Sigmodon hispidus*, into a normal rat we have been unable to find additional data concerning transplantation of adult filarial worms within a single species of mammal.

In the present investigation successful transplantation has been achieved in dogs as indicated by recovery of viable worms (av. length of female 29.8 cm.; average length of male 15.9 cm.) from the right heart of the recipient animals at least three weeks following transplantation.

Further information will be presented in a subsequent publication.

Facilities for the experiments were made available by Dr. C. A. Slanetz, Curator of Animal Husbandry, Columbia University Medical School.—PHILLIP H. MANN AND ITALO FRATTA, *Columbia University Medical School, New York.*

A PRELIMINARY REPORT ON THE LIFE CYCLE OF *CLOACITREMA MICHIGANENSIS* McINTOSH, 1938 (TREMATODA).

The trematode genus *Cloacitrema* was erected by Yamaguti (1934, Jap. J. Zool. **6**: 161–163). *Cloacitrema ovatum,* the type species, was found in the cloaca of *Bucephala clangula clangula,* from the Arctic Prefecture. The only other species in this genus is *Cloacitrema michiganensis* McIntosh (1938, Proc. Helminth. Soc. Washington **5**: 46–47) from the cloaca of the spotted sandpiper from Michigan and of the black-necked stilt from Florida.

Several years ago a number of specimens of *Cloacitrema michiganensis* were recovered from the cloaca of the spotted sandpiper, *Actitis macularia,* the western willet, *Catoptrophorous semipalmatus inornatus,* and the sea gull, *Larus californicus.* These birds were collected in the Playa del Rey region, Los Angeles County, along the coast of Southern California.

The eggs contain fully developed miracidia which may hatch in the uterus of the adult worm or in sea water. The miracidia are very active, swim rapidly and invade the snail host. Of the marine snails examined, only *Cerithidea californica* Haldeman was found naturally infected with the larval stages of this trematode. Two generations of rediae, mother and daughter, develop within the snail host. The cercariae are of the megalurous group and develop in the daughter rediae. They encyst as metacercariae on the surface of snail shells and on other objects. Two immature female sea gulls, *Larus californicus* were fed viable metacercariae and became infected with the adult flukes. The complete life cycle is the first recorded in the genus *Cloacitrema.*

Cloacitrema closely resembles *Parorchis* in structure and life cycle, both probably having an ancestor in common.

The morphology of the larval stages and a comparison of the life cycles of *Cloacitrema michiganensis* and *Parorchis acanthus* Nicoll, 1907 will be described in detail in a future report.—HENRY W. ROBINSON, *University of Southern California, Los Angeles, California.*

TEXAS RECORDS OF THE TICK, *IXODES TOVARI* COOLEY

The tick, *Ixodes tovari,* was described by R. A. Cooley in 1945 from a large series of specimens taken from "hares" in Nuevo Leon and Guanajuato, Mexico. It was first reported in the United States by Irons, et al. (1952, Jour. Parasit., **38**: 1–5). This record consisted of 2 nymphs from a domestic goat, taken by C. W. Johnson and O. L. Walker in Zavala County, Texas, May 11, 1950.

Subsequent *Ixodes tovari* records which have been obtained during Texas Q fever studies are: 1 female ex cottontail rabbit, *Sylvilagus auduboni,* Nov. 23, 1951, Uvalde County; 1 female ex *S. auduboni,* Nov. 28, 1951, Uvalde County; 1 nymph ex western meadow lark, *Sturnella neglecta,* Dec. 5, 1951, Zavala County; 1 nymph ex sparrow, *Amphispiza bilineata,* Uvalde County; 2 males ex *S. auduboni,* Dec. 21, 1951, Uvalde County; 1 female ex jackrabbit, *Lepus californicus,* Jan. 11, 1952, Uvalde County; 1 nymph ex cottontail, *S. auduboni,* Feb. 1, 1952, Uvalde County; 4 females ex *S. auduboni,* Feb. 4, 1952, Zavala County; 1 female ex *S. auduboni,* Feb. 7, 1952, Zavala County; and 1 larva ex *S. auduboni,* Feb. 8, 1952, Zavala County. All collections were made by O. L. Walker.

Definite conclusions relative to host preferences of the immature stages of this tick cannot be drawn from these records. However, the adults appear to primarily infest cottontail rabbits. There were numerous nymphs from Mexican "hares" in the material from which the original descriptions were made. In Texas, nymphs have been taken from domestic goat, western meadow lark and field sparrow, in addition to cottontail rabbit. A single larva has been taken from cottontail rabbit. The species is not too common in Zavala and Uvalde Counties since numerous rabbits, rodents, carnivores, domestic animals and birds are regularly examined in this area in connection with Q fever investigations and only the above records have been obtained.

Glen M. Kohls, Rocky Mountain Laboratory, Hamilton, Montana, Public Health Service, determined the first lot of *Ixodes tovari* collected, as well as several subsequent lots.—R. B. EADS AND O. L. WALKER, *Bureau of Laboratories, Texas State Department of Health, Austin.*

INCIDENCE OF *TRYPANOSOMA LEWISI, SARCOCYSTIS MURIS,* SPECIES OF SPIROCHAETA AND MICROFILARIAL LARVAE IN RATS IN PANAMA CITY AND SUBURBS

The problem of human trypanosomiasis has been extensively studied on the Isthmus of Panama since the first report on the disease [Miller, J. W.: Chagas Disease in Panama. Report of three cases, The Southern Med. Jour. **24:** 645 (July), 1931]. The same can be said about the equine trypanosomiasis, by *T. hippicum* Darling, 1910 [Darling, S. T.: Murrina. A Trypanosomal Disease of Equines in Panama, Proc. Canal Zone Med. Assoc. **3:** part 2, 47 (Oct.), 1910], and *T. vivax* Ziemann, 1905 [Johnson, C. M.: Bovine Trypanosomiasis in Panama, Amer. Jour. Trop. Med. **22:** 289 (March), 1941].

This paper reports the incidence of *T. lewisi* Kent, 1880, in rats in Panama City and suburbs, and, in addition, the incidence of *Sarcocystis muris* Blanchard, 1885, species of Spirochaeta and microfilarial larvae found through the examination of the blood of 351 rats, four of which were *Rattus rattus* and 347 were *R. norvegicus.*

The rats were supplied by the Gorgas Board of Health of the Canal Zone where they were previously killed and examined to determine their infection by the plague bacillus. We examined them, as a rule, from 24 to 72 hours after death. The author is thankful to Dr. N. W. Elton, Director of the Gorgas Board of Health, of the Canal Zone, for supplying us with the material which served as a basis for this study.

Our autopsy was limited to opening the thoracic cavity and removing the heart after severing the large arteries holding it. The heart was held between the legs of a tissue forceps, applied in a parallel manner at the height of the auricular-ventricular wall, under which the heart was dissected in order to separate the ventricles. The stump of the superior cardiac segment still being held by the tissue forceps, was used for the preparation of a thick smear, preferring an impression to friction, due to the fact that these smears showed fewer bacilli of putrefaction making the search for *T. lewisi* less difficult. However, we believe that this is the reason that our search for the leishmania forms of *T. lewisi* was negative. Whenever the blood expelled was not sufficient, the tissue forceps were moved one centimeter above the bloody surface, where sufficient blood was obtained for the preparation of the thick smear.

The staining of the slides was always made with Giemsa. No more than six slides were stained in the same jar to obviate the possibility, expressed by some, that the adult forms of *T. lewisi* might adhere to contiguous slides and give rise to false positives. The microscopic examination of each slide never took less than ten minutes. Although we cannot arrive at a conclusion regarding the incidence of *T. lewisi* and *S. muris* in the *R. rattus* species, due to the small number of specimens which were studied (four), we consider it worth mentioning that one of the rats was highly infected with *T. lewisi* and another one with *S. muris* spores.

In general, fifty rats were found infected with *T. lewisi,* which represents 14.5% of the total.

S. muris spores were found in 13 rats, which represents 3.8% of the total.

Seven rats were found infected with spirochaetes, which we did not try to identify, representing 2.0% of the total.

No microfilarial larvae were found in any of the rats.

Some reports have already been published on the incidence of *T. lewisi* in the rats of the American Continent, as shown on Table 1. Study of this table will show that the incidence

TABLE 1.—*Incidence of* Trypanosoma lewisi *in rats from the American Continent*

Author	City	Number examined	Number posit.	%
Price and Chitwood. 1931	Washington, D.C., U. S. A.	100	11	11.0
Andrews and White. 1936	Baltimore, U. S.A.	1515	167	7.4
Harkema. 1936	N. Carolina, U. S. A.	193	1	0.5
Cable and Headlee. 1937	Tippecanoe, U. S. A.	40	19	47.5
Herman. 1939	New York, U. S. A.	200	21	10.5
Beltran and Perez. 1947	Mexico City	500	18	3.6
Calero. This report	Panama City	351	51	14.5

varied from 0.5 to 47.5%, with an average of 13.4%. Our figure of 14.5%, therefore, leads us to believe that there is no hyper-parasitemia by *T. lewisi* in the rats of Panama City and suburbs.—CARLOS CALERO, M., *The Herrick Clinic, Panama Hospital, Panama, R. P.*